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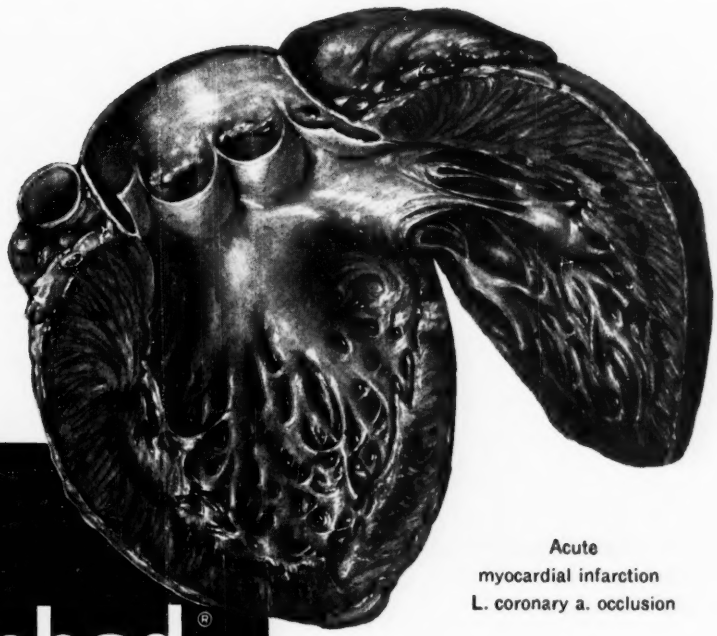

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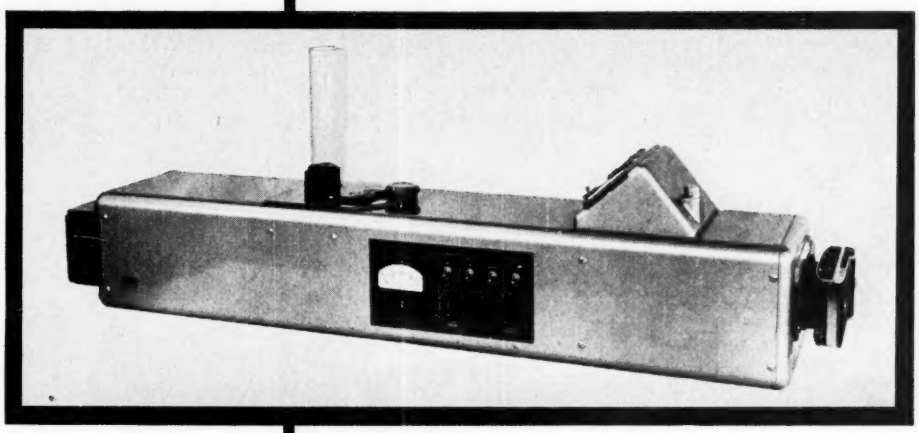

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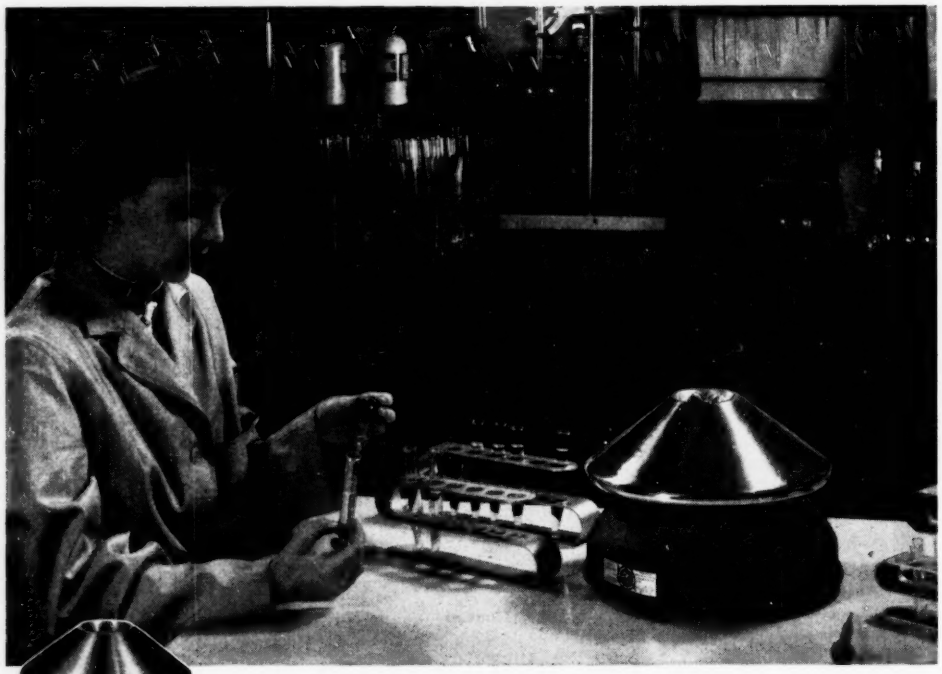

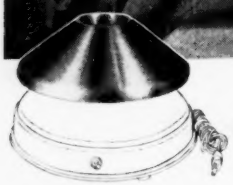

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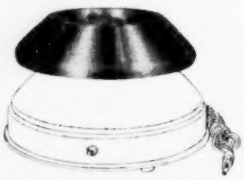

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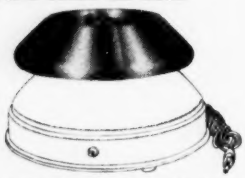

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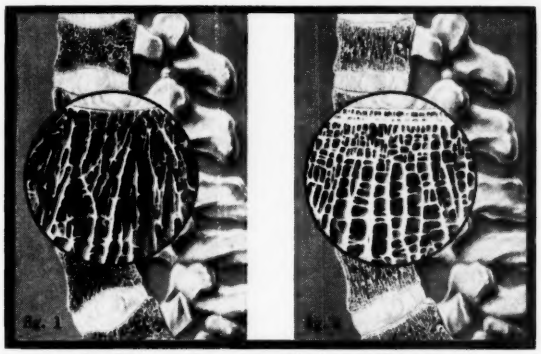

lumbar vertebrae, magnified sagittal sections

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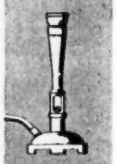

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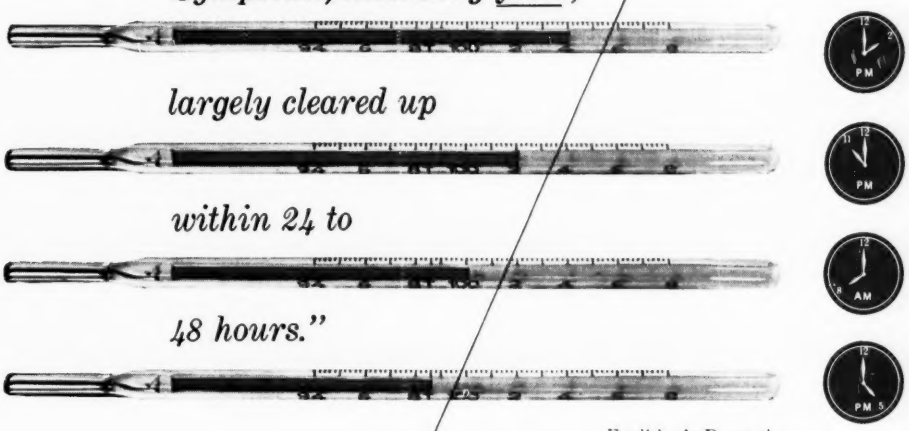

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CALORIGENIC EFFECTS OF SINGLE INTRAVENOUS DOSES OF L-TRIIODOTHYRONINE AND L-THYROXINE IN MYXEDEMATOUS PERSONS

By CHARLES M. BLACKBURN, WILLIAM M. McCONAHEY, F. RAYMOND KEATING, JR., AND A. ALBERT

(From the Section of Medicine, and the Section of Physiology, Mayo Clinic and Mayo Foundation,[1] Rochester, Minn.)

(Submitted for publication December 7, 1953; accepted January 27, 1954)

In 1950 and 1951 two unknown radioiodine-bearing compounds were observed (1–3) on chromatograms of plasma obtained from animals treated with I^{131}. Gross and Pitt-Rivers (4) identified one of these as 3,5,3′-l-triiodothyronine, which they had previously isolated (5) from the plasma of euthyroid and hyperthyroid individuals treated with I^{131}. They excluded the possibility of analytical or radiation artefact and considered 3,5,3′-l-triiodothyronine to be a normal constituent of the organic iodine moiety of plasma. Concomitantly, Roche, Lissitzky, and Michel (6) reported the conversion *in vitro* of diiodothyronine to triiodothyronine and crystallization of the latter compound. On the basis of its ability to prevent goiter in rats treated with thiouracil, Gross and Pitt-Rivers (7) found triiodothyronine three times as active as thyroxine. They also demonstrated its effectiveness (8) in raising the metabolic rate and correcting the symptoms of myxedema in two patients. In this respect they considered it to be at least as calorigenic as l-thyroxine and probably more so, and suggested that it might prove to be the tissue form of the thyroid hormone.

Since 3,5,3′-l-triiodothyronine is the only other amino acid to be found thus far which possesses hormonal properties comparable in intensity to those of thyroxine, it appeared desirable to make qualitative and quantitative comparison of the calorigenic properties of the two substances in human subjects.

METHOD

Eight persons with untreated myxedema who served as volunteers were hospitalized throughout the study. Duplicate determinations of the basal metabolic rate using the closed-chamber method were made daily except Sunday. Before the tests were made, the basal metabolic rate was determined daily for 6 to 16 days to establish a satisfactory base line. Serum-protein-bound iodine was estimated by the method of Barker (9).

Triiodothyronine and thyroxine[2] were administered in a small volume of sterile physiologic saline solution in which the substance was dissolved with the aid of sufficient sodium hydroxide. A single injection of the amino acid to be tested was given intravenously after the morning metabolic rate had been determined. Daily observations were then continued for as long as possible, in most instances until the curve depicting the basal metabolic rate had returned to a level within 1 or 2 calories per hour of the original base line. Careful record was made of clinical reaction and symptoms following the injections. Serum-protein-bound iodine was also determined at several intervals after injection.

In accordance with a procedure previously employed by other investigators (10, 11), mean daily metabolic rates were expressed as calories per day rather than as calories per hour. For each day following injection the mean base line obtained during the initial control period was then substracted from each daily value. The difference was considered to represent the increment in calories produced by the hormone being studied.

Each subject underwent two to six individual studies. In all, the effects of 18 doses of triiodothyronine and 11 doses of thyroxine were studied, involving a total of 1,213 determinations of basal metabolic rate. The data are summarized in Table I.

RESULTS

Following injection of either substance, the basal metabolic rate responded initially by rising to a peak value and subsequently by declining gradually toward the original base line (Figure 1). The decline of metabolic rate following injection of either drug could be fitted to a straight line when plotted on semilogarithmic paper versus time.

[1] The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

[2] These substances were provided through the courtesy of Smith, Kline & French Laboratories, who obtained the former from Glaxo Laboratories where it was synthesized.

TABLE 1

Summary of data on patients to whom triiodothyronine and thyroxine were administered *

Subject	Age, sex	Type of myxedema	Base line BMR	Base line PBI	Injection	Body weight, Kg.	Drug	Dose, μg.	T_{max}	C_{max}	$T_{\frac{1}{2}}$	C_{tot}	PBI 2 hr. after injection
1	35 M	Spontaneous	−34	0.5	1	56.5	T_4	333	7	80	—	1700	3.8
					2	57.1	T_3	333	2	220	7.2	1600	2.6
					3	57.6	T_3	160	3	112	9.7	1300	1.4
					4	58.8	T_4	3000	11	330	—	6700	14.3
2	45 F	Post I^{131} therapy	−26	1.9	1	72.2	T_3	200	2	300	7.0	2100	3.1
					2	72.4	T_4	300	7	145	10.8	2400	5.3
3	49 M	Spontaneous	−38	0.5	1	78.8	T_3	100	2	210	7.6	2100	0.7
					2	78.3	T_4	300	6	100	—	2100	1.7
					3	78.5	T_3	600	2	500	8.0	4600	2.6
					4	77.5	T_3	600	2	480	7.4	4100	—
					5	78.5	T_4	720	12	290	11.0	5000	5.6
					6	77.4	T_3	250	2	360	7.8	3300	0.9
4	58 M	Spontaneous	−33	0.6	1	81.5	T_3	100	2	195	7.7	1800	0.4
					2	79.4	T_3	100	1	200	7.7	1800	0.7
					3	79.8	T_3	600	3	320	8.2	3200	2.4
					4	79.0	T_3	400	2	290	7.7	2800	—
5	43 F	Spontaneous	−34	7.2†	1	60.9	T_3	100	2	190	7.9	1700	15.7
					2	61.1	T_3	600	2	375	7.7	2900	9.9
					3	60.5	T_3	100	2	190	7.8	1600	—
					4	61.3	T_4	800	13	164	11.4	4500	7.4
6	67 F	Spontaneous	−29	0.6	1	66.4	T_4	600	6	100	10.8	1900	3.5
					2	65.5	T_4	1800	9	240	—	5100	12.3
7	29 F	Spontaneous	−40		1	38.4	T_4	400	11	160	12.0	3300	—
					2	38.0	T_3	325	2	240	7.6	2100	—
					3	37.8	T_3	1000	3	270	7.6	2700	—
					4	37.6	T_4	1200	14	295	—	6600	—
8	52 F	Spontaneous	−27	Out of range‡	1	94.0	T_3	500	2	520	4.2	3300	Out of range‡
					2	92.8	T_3	1000	3	480	4.0	3100	—
					3	90.0	T_4	680	6	120	9.0	2100	—

* Abbreviations: BMR, basal metabolic rate in per cent; PBI, protein-bound iodine in micrograms per 100 cc.; T_{max}, time of maximal response in days; C_{max}, maximal caloric response in calories per day; $T_{\frac{1}{2}}$, half-value time in days; C_{tot}, total metabolic response in calories; T_3, triiodothyronine; and T_4, thyroxine.

† Recent cholecystogram.

‡ Recent excretory urogram.

With doses in excess of 4 micrograms per kilogram of body weight, triiodothyronine invariably produced subjective discomfort including malaise, restlessness, generalized aching, muscular and joint pain, loss of appetite, and headache. When the dose exceeded 10 micrograms per kilogram, these symptoms became intense. The symptoms accompanied the period of maximal metabolic response and, in most instances, subsided by the third or fourth day. These symptoms were identical with those often encountered in myxedematous patients whose metabolic rate is being rapidly elevated by administration of desiccated thyroid.

In contrast to the striking subjective discomfort following injection of triiodothyronine, similar symptoms did not occur after the administration of thyroxine in the doses here employed, although such symptoms have previously been observed to follow larger doses (10 mg. dl-thyroxine) given intravenously (10).

The curves depicting the effect of intravenous doses of each substance upon the metabolic rate were compared from four standpoints: (1) the time of maximal response (T_{max}), defined as the time in days elapsing between injection and greatest observed elevation of metabolic rate, (2) the half-value time ($T_{1/2}$), defined as the time in days required for the fitted curve of diminishing metabolic response (second limb) to reach half its value, (3) the maximal caloric response (C_{max}),

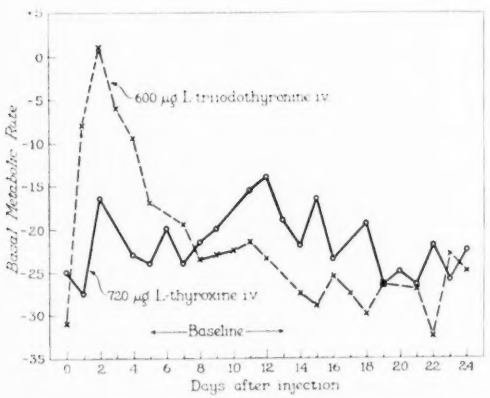

FIG. 1. THE BASAL METABOLIC RATES AFTER INJECTION OF EQUIMOLAR AMOUNTS OF TRIIODOTHYRONINE AND THYROXINE (SUBJECT 3)

defined as the maximal metabolic rate expressed in calories per day produced by a given injection, and (4) the total metabolic response (C_{tot}), defined as the total increment of increased energy production in calories throughout the time of action of the compound.

Following injection of triiodothyronine, the metabolic rate invariably rose more rapidly and to a higher maximal level than following equimolar doses of thyroxine (Table II). Maximal effects were always observed within 48 or 72 hours, the mean value for T_{max} being 2.2 ± 0.1 days. In contrast, thyroxine produced a slower and more sustained rise in metabolic rate, the maximal level being reached only after an interval of 6 to 13 days. The mean T_{max} for thyroxine was 9.4 ± 0.9 days. There was no correlation between dose and time of maximal response in the case of either drug.

The magnitude of the maximal response (C_{max}) reflected dosage and was always higher after triiodothyronine than after comparable doses of thyroxine. The dose-response relationships of the two substances in terms of this measurement are shown in Table II and Figure 2. Triiodothyronine was consistently more potent by a factor of 3 to 4.

The subsequent dissipation of calorigenic effect (thyroxine-decay curve of Boothby) was much more rapid after injection of triiodothyronine than after injection of thyroxine. Since in the case of either substance caloric response diminished exponentially, this rate could be expressed quantitatively either as a rate constant or as half-value time. Observed half-value times for triiodothyronine varied from 4.0 to 9.7 days (mean 7.4 ± 0.6 days) compared with corresponding values for thyroxine varying from 9.0 to 12.4 days (mean 11.9 ± 0.4 days). The latter values may be compared with those obtained in studies with thyroglobulin ("thyroiodin") made by Magnus-Levy in 1904 (12) which are calculated to have a half-value time of 15.6 days, and with the curves for dl-thyroxine published by Boothby and Baldes (13) and by other investigators (11) which are calculated to have half-value times of 10.8 days and 13.8 days, respectively. This difference in disappearance rate means that dose for dose the meta-

TABLE II

Comparison of peak values (maximal calories per day) and total calories resulting from similar doses of l-thyroxine and l-triiodothyronine

Subject	Thyroxine			Triiodothyronine		
	Dose, *millimols/Kg.* ($\times 10^6$)	Effect		Dose, *millimols/Kg* ($\times 10^6$)	Effect	
		Maximal cal./day	*Total calories*		*Maximal cal./day*	*Total calories*
1	7.6	90	1727	9.0	220	1585
2	5.4	145	2352	4.3	300	2120
3	5.0	100	2120	5.0	400	3300
	11.8	290	5020	11.8	480	4568
5	17.0	164	4800	15.1	375	2860
7	13.0	160	3300	13.0	270	2100
	40.0	295	6600	40.0	360	2700
8	8.0	120	2100	8.0	520	3300

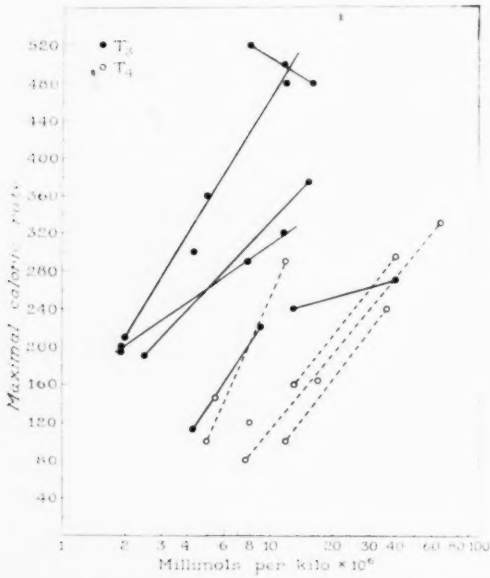

FIG. 2. DOSE-RESPONSE CURVES IN TERMS OF MAXIMAL CALORIES PER DAY FOR L-TRIIODOTHYRONINE AND L-THYROXINE

Solid lines connect points representing separate doses of triiodothyronine (T_3) given the same subject, and dotted lines connect points representing separate doses of l-thyroxine (T_4) given the same subject.

bolic effect of thyroxine long outlasts that of triiodothyronine. Seventy to 90 days are required for the dissipation of 99 per cent of the effect of a single injection of thyroxine compared to 45 to 55 days for the dissipation of 99 per cent of the effect of a single injection of triiodothyronine.

The half-value times, like the T_{max} values, were not correlated with dosage. There was some indication that variations in these values represented real differences in biologic response between individuals. If a given subject had a relatively short $T_{1/2}$ value for an initial study with triiodothyronine, subsequent studies also yielded low values, and more significantly, studies with thyroxine in the same subject disclosed low values for $T_{1/2}$ for this hormone also (see Subject 8, Table I).

Because of the difference in temporal relations observed with the two substances, the most significant basis for comparing their over-all effects appeared likely to be the total integrated caloric response (C_{tot}), represented by the total area under each curve of caloric response versus time. The area of the initial limb and the observed portion of the descending limb could be calculated by application of the trapezoidal rule. Since the descending limb of each such curve fitted a simple exponential function, it was possible to estimate a correction for the small residue of calorigenic activity remaining at the end of the period of observation and, in addition, for the residue of calorigenic activity remaining at the onset from any previous injection. Times of observation were arranged so that such corrections with few exceptions represented less than 10 per cent of the total area under each curve.

A dose-response curve constructed from such observations (Figure 3) indicated that when the dose was small the total caloric response resulting from an injection of l-triiodothyronine exceeded the response produced by an equimolar amount of l-thyroxine. However, with larger doses the reverse was true, that is, the total caloric response produced by thyroxine was greater.

The effect of the injections on protein-bound iodine can be judged from Table I. Thyroxine invariably produced a greater and more sustained elevation of protein-bound iodine than a comparable dose of triiodothyronine.

COMMENT

Since several successive injections were studied in each subject, it was necessary to be sure that

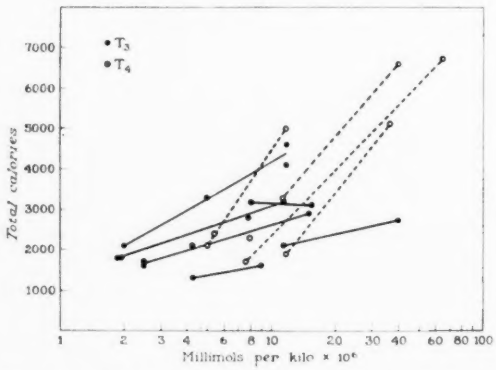

FIG. 3. DOSE-RESPONSE CURVES IN TERMS OF TOTAL CALORIES FOR L-TRIIODOTHYRONINE AND L-THYROXINE

Solid lines connect points representing separate doses of triiodothyronine (T_3) given the same subject, and dotted lines connect points representing separate doses of l-thyroxine (T_4) given the same subject.

subsequent responses were not essentially different from previous ones. For this reason duplicate studies were carried out in three subjects as shown in Table III and Figure 4. These studies indicate that results obtained with this method are reproducible and that successive doses may be evaluated accurately in the same subject providing sufficient time has elapsed between them. Investigators (11), in 1929, found a striking difference in the effectiveness of 10 mg. of intravenously administered racemic thyroxine at a basal metabolic level of − 40 per cent compared with the biologic effectiveness of the same dose at a metabolic level of − 4 per cent. However, they were comparing metabolic levels which differed by 20 or more calories per hour whereas in our study most comparisons were made at metabolic levels which did not differ by more than 1 calorie per hour.

In comparable doses, triiodothyronine was found to have an early and large maximal effect which disappeared rapidly whereas thyroxine exhibited a more delayed and less intense maximal effect which persisted for a longer interval. The more intense maximal effect of triiodothyronine frequently produced annoying subjective symptoms. These were not encountered with thyroxine except in doses much larger than those employed in this study. These qualitative differences posed practical obstacles to the bio-assay in man of small doses of thyroxine or large doses of triiodothyronine. Thyroxine could not be tested in doses of less than 300 micrograms because such doses did not produce sufficient elevation of the basal metabolic rate. Large doses of triiodothyronine could not be evaluated with safety because of the disturbing subjective symptoms. Therefore, single doses of the two drugs could be compared directly only in the dose range of 0.3 to 1.0 mg. Within this range of dosage the dose-response relationships of single injections of the two hormones have been compared in two ways. On the basis of maximal caloric response, triiodothyronine was found to be three to four times as potent as thyroxine. On the basis of total calories produced, the relative potency depended upon dose; with small doses, triiodothyronine was consistently more effective than thyroxine; with larger doses, thyroxine was more calorigenic.

TABLE III

Duplicate observations with triiodothyronine

Subject	Dose, μg.	Days between injections	Injection 1	Injection 2	Difference	
			Total calories		*Calories*	*Percent*
3	600	26	4568	4100	468	10.2
4	100	34	1790	1838	48	2.5
5	100	42	1673	1595	78	4.4

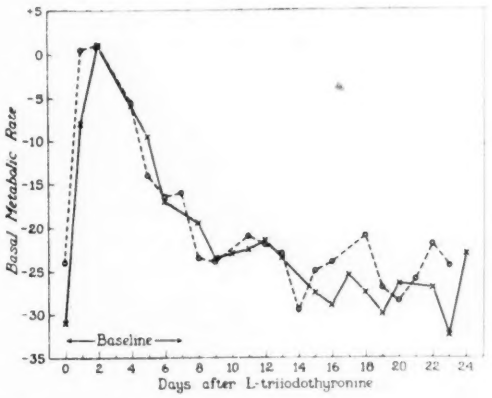

FIG. 4. METABOLIC RESPONSE TO INJECTION OF IDENTICAL AMOUNTS OF TRIIODOTHYRONINE ON TWO SEPARATE OCCASIONS (SUBJECT 3)

Valid comparison of the calorigenic potency of thyroidal substances may also be made on the basis of multiple doses, either by parenteral or oral routes. Salter, Lerman, and Means (14, 15) have employed an assay method based upon the administration of daily doses of the test substance over a period of two weeks or longer, accompanied by observation of the ascending limb of the resulting metabolic-response curve. This method could be subject to error if substances having major temporal differences in their responses were to be compared. A more secure comparison is one based upon the dose of each substance necessary to maintain a myxedematous subject in a steady euthyroid state over a period of time. Preliminary studies employing this technic would indicate triiodothyronine to be approximately two to three times more potent than thyroxine. The dose levels required for maintenance of a myxedematous subject are in the range 50 to 100 micrograms for triiodothyronine and 200 to 300 micrograms for thyroxine. This relationship agrees satisfactorily with that deduced from comparison of C_{max} values, and can also be inferred by extrapolation of the graph (Figure 3).

The changes which were observed in protein-bound iodine following injections of triiodothyronine and thyroxine could reflect more rapid destruction of triiodothyronine, a larger volume of distribution, or both, than in the case of thyroxine. Preliminary studies with radioactive triiodothyronine suggest that both phenomena are involved. A wider distribution of triiodothyronine could easily explain its more immediate calorigenic effect. If one accepts the premise that a hormone is metabolized in the course of exerting its effect, more rapid destruction would be expected, which might also be compatible with a shorter duration of action. These comparisons are compatible with the suggestion of Gross, Pitt-Rivers and Trotter (8) that triiodothyronine may be the tissue form of thyroid hormone, but do not provide any direct proof of such a theory.

SUMMARY

The calorigenic effect of single intravenous doses of l-triiodothyronine and l-thyroxine has been compared in eight subjects having myxedema. Following administration of l-triiodothyronine the basal metabolism rose more quickly to a maximal value, and subsequently fell toward the base line more rapidly than following administration of l-thyroxine. The mean time elapsing between injection and maximal metabolic rate was 2.2 ± 0.1 days in the case of the former and 9.4 ± 0.9 days in the case of the latter. After a maximal value was reached, both curves decreased exponentially, the half-value time for triiodothyronine being 7.4 ± 0.6 and for thyroxine 11.9 ± 0.4 days. When compared on the basis of the maximal metabolic rate resulting from a given injection, triiodothyronine appeared to be three or four times as potent as thyroxine. On the basis of total calories added to the base line throughout the duration of action of each injection, the dose-response curves of the two substances were not parallel. On this basis, with small doses, triiodothyronine was more calorigenic than thyroxine but with larger doses (above about 10 micrograms per kilogram of body weight) thyroxine was more effective than triiodothyronine.

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STUDIES ON INTRAVASCULAR HEMOLYSIS IN MAN. THE PATHOGENESIS OF THE INITIAL STAGES OF ACUTE RENAL FAILURE

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Intravascular hemolysis of moderate or severe degree in man is often followed by acute renal failure. Most studies of renal function have been carried out on patients during or after the oliguric or anuric phase and have, therefore, shed little information on pathogenesis of the renal failure.

The present studies were undertaken to clarify the changes in renal function which precede anuria resulting from intravascular hemolysis. It was hoped that the onset of acute renal failure might be prevented if the means of reversing the early changes could be found.

The changes in renal function in 10 patients during intravascular hemolysis induced by the intravenous infusion of distilled water are described in this report. The infusions were given slowly, carefully controlled, and often continued for many hours. The resulting alterations in renal function rapidly returned toward normal after the infusion of water was discontinued or after the intravenous injection of Parathormone.

The initial justification for this study rested on observations of the changes in renal function in a patient during and after the accidental intravenous infusion of distilled water. She developed anuria but urine flow was reestablished and her inulin clearance returned to normal 30 minutes after the intravenous injection of Parathormone. Twelve weeks later her inulin clearance was 140 ml. per min. per 1.73m², Diodrast clearance 420 ml. per min. per 1.73m², but Tm Diodrast was only 11 mg. per min. per 1.73m². Clinical assessment of her renal function, including urine concentration tests, during the next three years has revealed no abnormality.

Patients with impaired renal function, as shown by a urine concentration test and routine clinical tests, were excluded from the present study. A urine specimen of specific gravity of at least 1020 was required before subjects were accepted for study.

At first the only patients studied were those with Hodgkin's disease, lymphosarcoma, or leukemia who were receiving X-ray treatment. Some patients without malignant disease were studied after the experimental procedures proved safe. Two of our experimental patients died some months after the studies were completed, of lymphatic leukemia and lymphosarcoma, respectively, and autopsy examinations revealed no renal abnormality which could be attributed to intravascular hemolysis.

Many investigators have failed to demonstrate renal failure during or after the infusion of hemoglobin solutions in man and experimental animals unless other abnormalities were present, for example hypotension (Rosoff and Walter [1]) or anaphylaxis (Mason and Mueller [2]). Landsteiner and Finch (3) and Voris (4) gave massive intravenous infusions of distilled water but described no instance of renal failure though detailed studies of renal function were not reported. Amberson, Jennings, and Rhode (5) reported oliguric reactions twice in a series of 77 infusions of hemoglobin solutions. On the other hand, Miller and McDonald (6) regularly demonstrated changes in renal and cardiovascular function in 26 normal subjects following the infusion of pure hemoglobin solutions and of freshly lysed red blood cells. The latter authors did not report any severe or permanent renal damage following their experimental procedures.

METHODS

Subjects: Nine of the 10 experiments were carried out on patients receiving X-ray therapy for leukemia, lymphosarcoma, or Hodgkin's disease. Some data regarding these subjects are set out in Table I.

[1] Medical Board Fellow in Medicine, 1951.

[2] Supported in part by a Grant from the National Health and Medical Research Council.

Design of experiments: Inulin and Diodrast clearances were measured by the standard techniques of Goldring and Chasis (7) in the fasted but continuously hydrated patient. The inulin and Diodrast infusion, made up in 4 per cent glucose-water, entered a vein in one leg and an infusion of 4 per cent glucose-water was run into a small vein of the opposite ankle or foot. Both these infusions were given at a rate of 4 ml. per minute, a total of 8 ml. per minute. Venous blood specimens were collected from one arm and measurements of blood pressure were made by sphygmomanometry on the other arm. Catheterization of the bladder, starting the infusions and general setting-up for the experiments usually took place 1 to 2 hours before the control observations were made. After two to five control periods distilled water was substituted for the 4 per cent glucose-water infusion and was usually given at the same rate of 4 ml. per minute. After discontinuing the distilled water infusion glucose-water was again given to maintain a constant intravenous infusion rate. Clearance periods were usually 15 to 40 minutes but were prolonged when oliguria was pronounced. Blood pressures, pulse rates, and oral temperatures were usually recorded each 20 to 30 minutes. No significant changes were observed in venous pressures which were recorded in antecubital veins with a saline manometer.

Chemical methods: Inulin was determined by Little's (8) method, Diodrast by Alpert's (9) method, and urine hemochromogen concentration by Bing and Baker's (10) method. Plasma hemochromogen concentrations were estimated by the method of Hunter, Grove-Rasmussen, and Soutter (11) so that determinations could be made each few minutes during the experiments. All clearances have been corrected to a standard surface area of 1.73 square meters.

Renal vascular resistances: Resistances were calculated from the equation $R = \frac{Pm \times 1328}{RBF}$ where R = renal vascular resistance, Pm = half the sum of the values for the systolic and diastolic arterial blood pressures and RBF = the renal blood flow in ml. per second calculated from the Diodrast clearance and the hematocrit value.

Hemoglobin solutions were prepared from an appropriate volume of the patient's own blood, say 300 ml., collected 24 to 30 hours prior to the experiment. Preparation of the cell and stroma free solution was by standard methods involving red cell washing, freezing and thawing, centrifuging and Seitz filtering. All operations were carried out at 4° C. Concentrations were adjusted in the final saline-hemoglobin solution so that a rate of infusion of 4 ml. per minute could be continued without lowering the plasma hemochromogen concentration.

Parathormone (Eli Lilly & Co.) for intravenous injection was either the freshly delivered solution, or solutions which were relatively inactive as the result of autoclaving or of being stored for many months beyond the maker's date of expiration. On two occasions an infusion of Parathormone in 4 per cent glucose-water was used instead of a single intravenous injection. The type of Parathormone preparation injected made no significant difference to the patient's response with regard to the renal functions considered in this paper (12).

RESULTS

The detailed results of the studies on 10 patients are set out in Table II. Seven developed significant oliguria during the water infusion. The infusions were continued for many hours and were usually stopped when the patients began to show evidence of adverse psychological reaction such as restlessness and distress from hunger, fatigue, and stiffness in immobilized limbs. Sedation with barbiturates was always minimal.

In these 10 patients, age, sex, the nature of the disease process and the previous experience of blood transfusion appeared to have no influence on the occurrence of an oliguric reaction.

Systemic effects were minimal. The arterial pressure rose slightly in two patients one of whom was hypertensive before the experimental study. Transient, variable and minor changes in blood pressure were attributed to inadequacies of standard sphygmomanometry and to psychological reactions rather than to intravascular hemolysis. Five of the patients had febrile reactions during

TABLE I

Clinical data on 10 patients who received intravenous infusions of distilled water

Subject	Sex	Age	Diagnosis	Hematocrit	Oliguric reaction
1. PAY	M	36	Chronic myeloid leukemia	0.43	+
2. PAT	M	35	Acute myeloid leukemia	0.40	+
3. ELL	M	43	Lymphosarcoma, Hypertension	0.48	+
4. FER	M	52	Lymphosarcoma	0.50	+
5. SMI	M	31	Hodgkin's disease	0.44	+
6. NEE	F	43	Chronic myeloid leukemia	0.37	+
7. FLO	M	32	Hodgkin's disease	0.47	+
8. QUE	M	41	Lymphosarcoma	0.37	−
9. HUG	M	55	Chronic lymphatic leukemia	0.38	−
10. SOF	M	26	"Neurasthenia"	0.46	−

or after the experiments ended and the following elevated oral temperatures were recorded: 38.4° C in PAT 20 minutes after the study ended and persisting for 6 hours; 38.6° C in SOF 30 minutes after the study ended and persisting for 2 hours; 38° C in PAY 1 hour before the study ended and persisting for 1.5 hours; 38.2° C in QUE in the last period, reaching 39.2° C 1 hour later but falling in the next 2 hours; 38° C in SMI at the end of the study, 39.6° C 1 hour later and falling to less than 38° C 3½ hours after the study ended. These reactions were attributed to the manipulations of the experiments and to the presence of traces of pyrogens in the infusions which had been given for many hours.

The manifestations of "transfusion reaction" were completely absent. Backache was never more than mild and, presenting with a slow onset after some hours of flat recumbency, was not unexpected.

Oliguria developed in the same manner in all of the seven patients who showed it. Urine flow started to diminish shortly after the infusion of water was begun and continued to decrease until steps were taken to increase it. In four patients, at the depth of oliguria, the urine volume was virtually zero during 20 to 30 minute periods. Progressive oliguria was always associated with progressive decrease in Diodrast clearance, with a fall in inulin clearance in six patients, and a rise in filtration fraction in four patients. Hemoglobinuria usually accompanied or immediately followed significant oliguria.

Validity of renal clearances: Inulin and Diodrast clearances were considered to indicate glomerular filtration rate and renal plasma flow, respectively. Extraction ratios were not performed but normal ratios were assumed since Miller and McDonald (6) found normal ratios in similar experiments. Other investigators (13) clearly demonstrated that inulin and PAH clearances were valid in the dog until the renal blood flow fell to about 3 per cent of normal, a decline far greater than we ever observed. Finally the changes in renal function in our patients were rapidly reversible and U/P ratios for inulin always rose as urine volume decreased.

Collection periods of 20 to 40 minutes are too short for the accurate measurement of urine flows of 0.5 ml. per minute and less and the ureteropelvic dead space is large in comparison with the collected urine volume. Clearances calculated for the periods of minimum urine flow will be too low whilst those for the periods immediately following will be too high. Renal storage and release of Diodrast may exaggerate the errors in the calculation of some clearances. In spite of these errors the continuous series of clearances are taken to indicate the magnitude and direction of the changes in filtration rate and renal plasma flow in each patient.

Diodrast clearances: A highly significant direct correlation developed between the rate of urine flow and Diodrast clearance during the infusion of distilled water. Prior to the infusion of distilled water the coefficient of correlation was low and not significant ($r = 0.08$, $n = 30$ and $P = > 0.1$) but during the infusion of distilled water the correlation coefficient was higher and highly significant ($r = 0.54$, $n = 41$ and $P < 0.001$). Correlation coefficients were derived from the logarithms of the data obtained from all 10 patients during the control period and during the infusion of distilled water but the data obtained during or after the infusion of Parathormone were excluded.

Renal plasma flow was reduced in the seven patients who developed oliguria during the in-

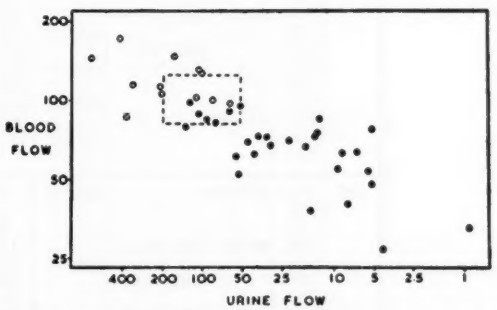

FIG. 1. RELATIONSHIP BETWEEN THE CHANGE IN RENAL PLASMA FLOW (C_D) AND THE CHANGE IN URINE FLOW IN 10 PATIENTS DURING THE INTRAVENOUS INFUSION OF DISTILLED WATER

C_D and UV are both plotted as percentages of the mean control values (100 per cent) for each patient. The area included by the dotted lines represents the mean $\pm 2\sigma$ of all the control C_D and UV values. Solid circles represent the data from the seven oliguric patients, the closed circles the data from the three patients without oliguria.

TABLE II

The effects of intravenous infusions of distilled water on the renal functions of 10 patients

SUBJ.	TIME	INTRAVENOUS INFUSION	B.P.	P.Hb	U.Hb	UV	C_{in}	FF	C_d	RENAL RESISTANCES
PAT	0-15	Glucose	110/70		-	12.00	144	.23	630	6500
	41	"			-	18.25	115	.18	629	6150
	78	Water (4ml/min)		66	-	6.49	86	.20	422	9200
	111	" "	100/60	102	-	lost				
	150	" "		138	+	negligible urine, added to next				
	185	Glucose at 170	110/70	179	+	0.14	71	.35	202	20200
	220	Glucose		143	+	0.43	86	.32	269	14350
	255	"	100/60	105	+	4.00	157	.30	528	6850
	290	"		93	+	9.72	146	.30	492	7600
	327	"	110/60	70	+	8.29	144	.30	495	7800
	362	"	100/70	58	+	7.85	185	.33	566	6800
	396	"		42	+	6.61	119	.18	661	5850
PAT	0-17	Glucose	125/70		-	4.53	91	.16	573	8150
	34	"			-	9.55	91	.18	502	9550
	54	"	130/75		-					
	78	"		2	-	7.00	89	.18	494	9900
	101	Water (4ml/min)	130/85	17	-	8.70	79	.15	510	10100
	123	" "	130/80	66	-	3.63	56	.18	318	15800
	154	" "		131	-	0.97	29	.15	195	26950
	178	" (2ml/min)	150/80	133	+	negligible urine, added to next				
	197	" "		129	+	0.33	69	.17	400	13750
	221	" "		128	+	0.79	93	.21	439	
	244	Glucose		124	+	2.70	95	.20	467	
	266	"		101	+	5.19	71	.21	334	
ELL	0-17	Glucose	170/105	10	-	3.47	103	.28	368	15450
	36	"		16	-	6.31	140	.35	395	14150
	54	"	160/105	10	-	7.94	140	.37	379	14450
	79	Water (4ml/min)		16	-	7.60	93	.31	300	21100
	102	" "		49	-	4.96	97	.30	319	19900
	130	" "	180/110	81	-	2.07	76	.28	276	21750
	146	" "		101	+	0.88	67	.34	195	32500
	164	" " + PT		115	+	3.33	156	.31	498	12750
	187	" "	170/100	122	+	10.88	87	.25	353	15850
	210	" "	180/105	140	+	7.48	91	.25	362	16300
FER	0-19	Glucose	140/90	7	-	5.68	75	.15	510	9000
	43	"		2	-	6.00	88	.17	504	9100
	67	Water (4ml/min)		65	-	2.37	72	.23	312	14650
	88	" "	140/90	135	-	0.62	95	.34	277	16500
	110	" (2ml/min)		151	+	0.77	140	.37	381	12000
	137	" (3ml/min)	140/90	170	+	1.22	126	.36	351	13000
	159	" "		192	+	0.50	94	.48	198	23100
	175	" "	140/90	180	+	0.31	62	.46	136	34600
	207	Glucose +PT		165	+	4.35	174	.25	658	6950
	236	"	140/90	140	+	3.15	117	.26	452	10100
	267	"			+	0.97	155	.35	445	10300
SMI	0-18	Glucose	100/70	5	-	3.72	105	.18	573	6600
	35	"			-	9.17	131	.16	798	4450
	56	"		8	-	9.71	84	.14	596	6000
	84	Water (4ml/min)		13	-	8.65	70	.13	528	8750
	106	" "	90/60	46	-	3.63	54	.16	332	10100
	128	" (1ml/min)		51	-	0.36	52	.15	341	9800
	152	" "		57	-	0.46	64	.16	407	8200
	174	" (4ml/min)		67	-	negligible urine, added to next				
	198	" (1.5ml/min)	90/60	70	-	0.33	59	.19	304	11000
	222	Glucose + PT		61	+	2.38	99	.14	729	4600
	242	"		52	+	2.55	98	.13	737	4500

TIME in minutes
B.P. = blood pressure in mm of Hg
P.Hb = plasma haemochromogen in mg %
U.Hb = haemoglobinuria
UV = urine volume in ml / min
Cin = inulin clearance in ml / min
FF = filtration fraction
Cd = diodrast clearance in ml / min
RENAL RESISTANCES in dynes sec cm^{-5} x 10^{-3}
Glucose = 4% glucose-water at 4 ml / min
Water = distilled water
PT = injection of 200U 'Parathormone'

TABLE II *Continued*

SUBJ.	TIME	INTRAVENOUS INFUSION	B.P.	P.Hb	U.Hb	UV	C_{in}	FF	C_d	RENAL RESISTANCES
NEE	0-22	Glucose	105/70	5	-	2.36	129	.26	496	8900
	49	"	115/80	6	-	0.70	70	.12	581	8450
	72	"	120/80	6	-	0.86	84	.21	403	12450
	98	Water (4ml/min)			-	0.58	73	.16	466	9700
	120	" "	110/70	17	-	negligible urine, added to next				
	144	" "		42	-	0.33	53	.16	325	13300
	166	" " + PT infusion	105/60		-	5.56	331	.15	2220	1870
	188	" " + PT infusion		135	+	1.41	106	.10	1056	4100
	211	" " + PT infusion	110/70	136	+	0.48	75	.12	650	6160
	234	Glucose	110/70	134	+	0.22	67	.15	443	10200
	247	"	110/70		+	0.46	155	.12	1315	3430
FLO	0-16	Glucose		5	-	8.87	104	.18	570	6700
	34	"		3	-	12.75	107	.22	492	7700
	54	"		4	-	15.10	93	.18	528	7200
	77	"		4	-	16.33	90	.17	537	7100
	95	Water (4ml/min)		45	-	14.30	106	.23	463	8200
	110	" "	105/75	72	-	9.86	91	.19	472	8050
	130	" "		133	-	4.15	79	.21	379	12250
	156	" "		166	+	1.77	92	.24	379	12250
	168	" "	115/75	197	+	0.25	78	.32	243	16500
	185	Hemoglobin		226	+	0.94	101	.48	211	18800
	206	"	110/75	212	+	3.33	117	.28	414	9450
	227	"		218	+	7.24	107	.35	310	12800
	249	"	115/75	226	+	10.61	149	.47	317	12700
QUE	0-15	Glucose	125/85	8	-	1.33	124	.23	539	9800
	29	"	120/80	10	-	0.86	136	.27	503	10000
	52	"	130/90	5	-	1.39	131	.18	716	7750
	78	"	125/85	5	-	2.11	126	.17	780	6750
	108	"	105/80	9	-	1.50	76	.12	646	7200
	133	Water (4ml/min)	105/75	23	-	0.76	85	.14	607	7450
	158	" "		44	-	1.12	112	.18	630	7400
	183	" "	110/80	63	-	2.80	99	.15	652	7300
	209	Glucose + PT		73	-	3.38	98	.12	800	6150
	234	Glucose	120/80	70	-	4.68	100	.12	882	5700
	260	"	110/80	55	-	6.35	112	.13	844	5650
	285	"	120/80	54	-	7.00	114	.15	738	6800
SOF	0-15	Glucose	110/90		-	2.40	82	.20	407	12100
	34	"	110/90	3	-	1.58	102	.20	503	9800
	59	Water (4ml/min)	105/85	25	-	8.00	127	.16	775	6050
	85	" "	120/95		-	14.30	112	.17	651	8150
	109	" "	120/95	90	+	6.58	110	.22	505	10500
	130	" "	125/95	104	+	7.15	83	.22	386	13750
	154	" " + PT infusion	120/90	125	+	10.82	96	.14	696	7450
	175	" " + PT infusion	125/95		+	16.19	92	.11	823	6450
	197	" " + PT infusion	130/80	146	+	11.37	85	.12	711	7300
	219	Water (4ml/min)	120/80	148	+	8.18	103	.13	810	6100
	242	" "	120/85	140	+	2.17	77	.12	620	8150
	266	" "	115/85	130	+	1.13	101	.16	620	7950
HUG	0-20	Glucose	125/65	3	-	3.80	50	.14	363	11250
	43	"		7	-	1.65	72	.15	466	8750
	67	"		3	-	1.96	79	.18	430	9500
	90	Water (4ml/min)		15	-	3.57	56	.11	530	7700
	115	" "	125/65	62	-	4.32	66	.14	468	8700
	141	" "		77	-	2.31	88	.17	530	7700
	163	" "		88	-	2.41	89	.17	540	7550
	180	" "		106	-	2.47	77	.18	425	9600
	205	" " + PT		103	-	13.90	120	.16	735	5550
	231	" "	125/65	111	-	12.70	92	.19	475	8600
	258	" "		114	-	11.28	107	.24	440	9250
	283	" "	125/70	124	-	10.20	98	.27	362	11600

fusion of water and this reduction started as soon as urine flow began to decrease that is, when hemolysis began.

Inulin clearances: A significant direct correlation developed between the rate of urine flow and inulin clearance during the infusion of distilled water though it was not demonstrable during the control periods. Correlation coefficients were derived in the same manner as for Diodrast clearances and were similar. Prior to the distilled water infusion the correlation coefficient was low and insignificant ($r = 0.14$, $n = 30$, $P = > 0.1$) but during the infusion of distilled water the correlation coefficient was higher and highly significant ($r = 0.41$, $n = 41$, $P = < 0.01 > 0.001$).

The correlation between GFR and urine flow was not as close as that between urine flow and RPF (Table II, and Figure 2).

Filtration fraction: This function rose in four patients and was considerably increased during the period of maximal oliguria. The maximum figures in the control periods were 0.23, 0.18, 0.17 and 0.22 but in the experimental periods 0.35, 0.21, 0.48, and 0.32 respectively.

Calculated renal vascular resistances: These resistances were increased in all patients developing oliguria and were considered to indicate renal vasoconstriction.

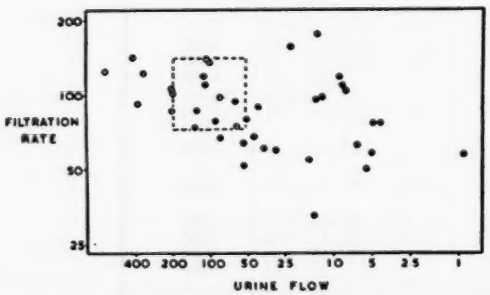

FIG. 2. RELATIONSHIP BETWEEN THE CHANGE IN GLOMERULAR FILTRATION RATE (C_{IN}) AND THE CHANGE IN URINE FLOW IN 10 PATIENTS DURING THE INTRAVENOUS INFUSION OF DISTILLED WATER

C_{IN} and UV are both plotted as percentages of the mean control values (100 per cent) for each patient. The area included by the dotted lines represents the mean $\pm 2\sigma$ of all the control C_{IN} and UV values. Solid circles represent the data from the seven oliguric patients, the closed circles the data from the three patients without oliguria.

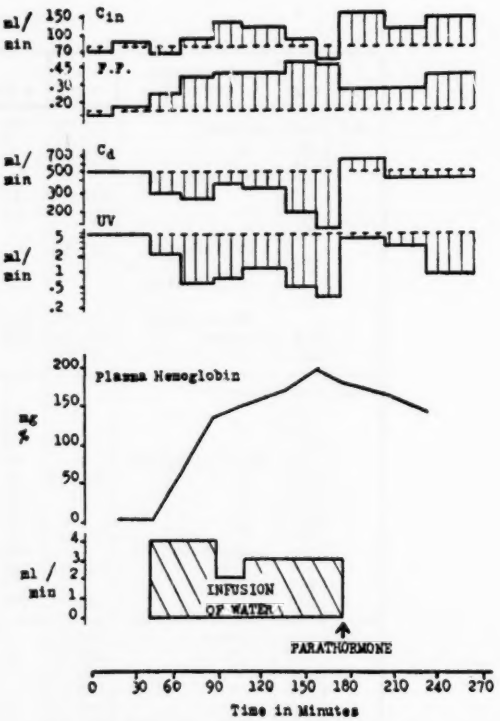

FIG. 3. CHANGES IN RENAL FUNCTION AND PLASMA HEMOCHROMOGEN CONCENTRATION DURING AND AFTER THE INFUSION OF DISTILLED WATER (SUBJECT FER)

Alterations in renal function bear an inverse relation to the rate of water infusion and are promptly restored towards normal by the injection of 200 units of Parathormone intravenously.

Plasma and urine hemochromogens: The plasma hemochromogen concentration bore no constant relation to the development of oliguria, the levels were no higher in the patients developing oliguria than those who did not. The amount of hemolysis depended mainly on the size of the vein receiving the infusion and on the blood flow through it as the infusions of water were usually given at the same rate, 4 ml. per minute. In general, the smaller the vein used the greater the hemolysis. The highest plasma hemochromogen concentrations were from 70 to 226 mg. per cent with a mean of 142 mg. per cent.

The rate of infusion of water appeared to be of greater importance in producing changes in renal function than the plasma hemochromogen concentration, for decreasing the rate of water infusion

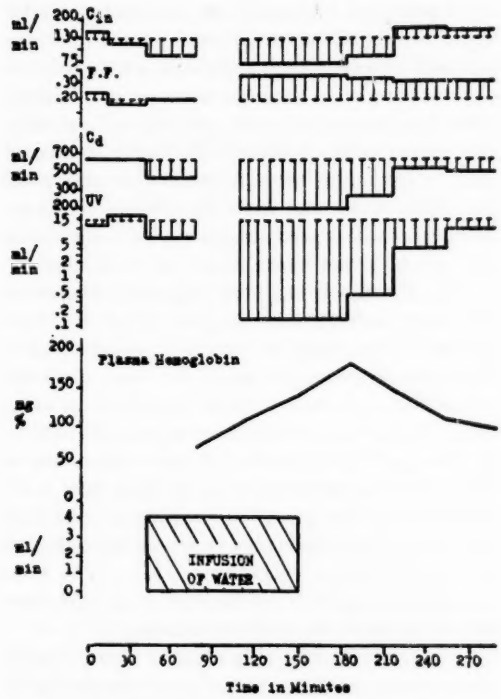

FIG. 4. CHANGES IN RENAL FUNCTION AND PLASMA HEMOCHROMOGEN CONCENTRATION DURING AND AFTER THE INFUSION OF DISTILLED WATER (SUBJECT PAY)

resulted in a prompt increase in urine flow and Diodrast clearance (PAT, FER, SMI in Table II and Figure 3). Increasing the rate of water infusion produced the reverse effects. The changes in plasma hemochromogen concentration after it became elevated were quite minor in degree and only once did the concentration fall after a temporary decrease in the rate of infusion of water.

Oliguria was accompanied or followed by hemoglobinuria in six of the seven patients developing concomitant decreased renal plasma flow, but hemoglobinuria was observed in the absence of oliguria or diminished renal blood flow (SOF in Table I). Hemoglobinuria usually appeared when the plasma concentration was 100 to 150 mg. per 100 ml.

Changes after stopping the distilled water infusions: In the oliguric patients urine flow, renal plasma flow and filtration rate increased in the first period after stopping the distilled water infusion and then returned toward, or to, the control levels (PAY and PAT in Table II, Figure 4). This reversal was significant within 20 minutes. A similar effect was observed when a solution of the patient's own stroma free hemoglobin (0.7 per cent) in 0.85 per cent saline was substituted for distilled water (FLO in Table II, Figure 5). This reversal took place in the presence of unchanged or increased plasma hemochromogen concentrations and often in the presence of increased hemoglobinuria.

Effects of Parathormone: The intravenous injection of Parathormone solution produced a prompt diuresis, a marked increase in Diodrast clearance and a fall in filtration fraction in every patient who was given it whether they had oliguria or not (ELL, FER, SMI, NEE, QUE, SOF, HUG in Table II, Figures 3 and 6). In five of these seven patients inulin clearances increased considerably but in two (QUE, SOF)

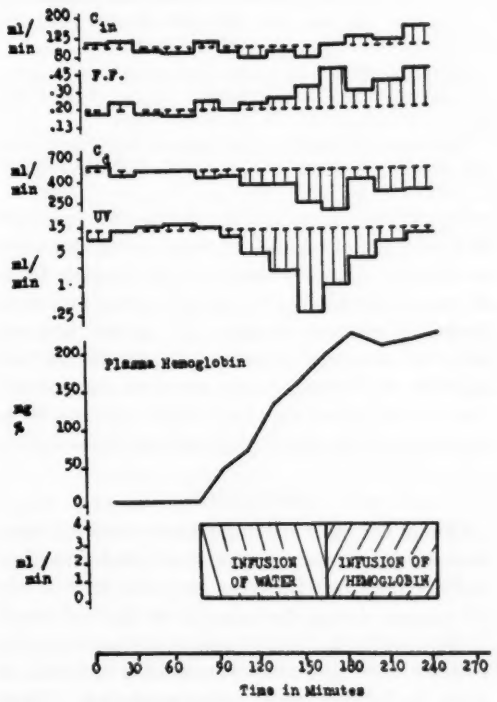

FIG. 5. CHANGES IN RENAL FUNCTION AND PLASMA HEMOCHROMOGEN CONCENTRATION DURING THE INFUSION OF DISTILLED WATER AND DURING THE INFUSION OF A SOLUTION OF THE PATIENT'S OWN HEMOGLOBIN (SUBJECT FLO)

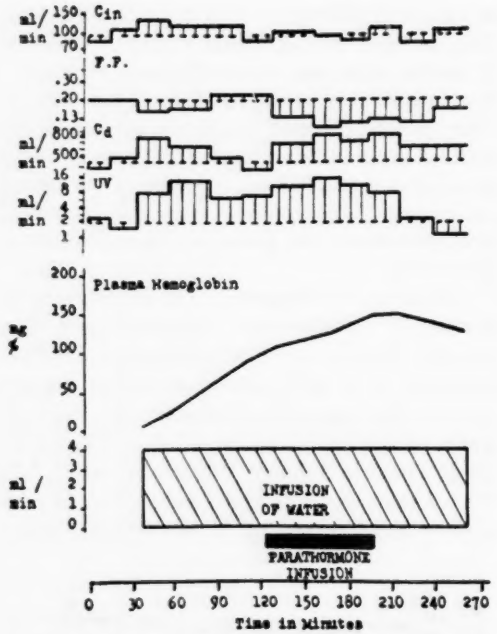

FIG. 6. CHANGES IN RENAL FUNCTION, AND PLASMA HEMOCHROMOGEN CONCENTRATION DURING THE INFUSION OF DISTILLED WATER (SUBJECT SOF).

Intravenous injection of 200 units of Parathormone in 100 ml. saline produce prompt changes in C_D and UV.

were unchanged and neither of these two patients had evidence of significant renal vasoconstriction or oliguria. These reactions were typically those of intense renal hyperemia and were unaccompanied by systemic changes. No patient had evidence of abnormal sensitivity to the intradermal injection of Parathormone prior to the intravenous injection and the renal effects appeared to be unrelated to any effect on phosphate reabsorption.

DISCUSSION

One of the most striking changes in renal function was the occurrence of a diminished urine flow and a diminished Diodrast clearance in 7 of the 10 patients during the infusion of distilled water. A diminished Diodrast clearance was considered to indicate decreased renal plasma flow and this, in turn, to indicate renal vasoconstriction. Renal vasoconstriction did not appear to be a part of gross generalized vasoconstriction in our patients for there were no significant changes in arterial blood pressure or pulse rates. Miller and McDonald (6), on the other hand, recorded raised blood pressures in normal subjects during similar experiments. A decreased renal plasma flow was not only observed in the patients with profound oliguria but also in three patients whose minimum urine flows were 0.25, 0.31, and 0.88 ml. per minute, respectively. A statistically highly significant direct correlation between urine flow and renal blood flow was found when the data on all 10 patients were considered though considerable variation was observed in the data from individual patients. Though it has been suggested that urine flow may be influenced by renal blood flow to a greater extent than is usually considered (14) significant correlations have not been recorded unless there has been a great reduction in urine flow. Urine flow does vary with renal blood flow in the experimental animal if the renal artery is temporarily occluded (15), or if blood flow is reduced though the perfusion pressure be kept constant (16), or if the kidney is previously subjected to 5 to 20 minutes of ischemia (17). The renal functional changes in our patients resembled those of post-ischemic or perfused kidneys.

Changes in the rate of infusion of distilled water were promptly followed by inverse changes in the rates of urine flow and renal blood flow in oliguric patients. Renal function returned toward normal when the distilled water infusion was stopped and 4 per cent glucose in water substituted for it.

The effects of distilled water did not appear to be due to impurities since the same water was used for making up all the intravenous solutions given to these patients in the three-year period during which the studies were made. Some patients did develop pyrexial reactions but there was no correlation between these reactions and the development of oliguria or diuresis.

Intravenous infusion of distilled water led to hemolysis and some product of hemolysis may have caused renal vasoconstriction and oliguria. Hemoglobin and its degradation products, unknown vasoconstrictive substances and erythrocyte stroma have all been suggested as proximate causes of acute renal failure.

It is difficult to attribute decreased renal blood flow and oliguria directly to the effects of raised plasma hemoglobin levels. One patient had a diuresis and increasing renal blood flow, in spite of a rising plasma hemochromogen level, when a 0.7 per cent solution of his own hemoglobin in 0.85 per cent saline was substituted for distilled water.

The appearance of renal vasoconstriction and oliguria appeared not to depend on the plasma hemochromogen concentration nor did urine flow vary inversely with it except in a very general way. Hemoglobin solutions have been infused into convalescent patients and patients with renal disease to determine glomerular permeability and, though plasma hemoglobin levels of 500 mg. per cent were reached, no instance of oliguria or anuria was recorded (18). Badenoch and Darmady (19) concluded that hemoglobin was nephrotoxic only if a critical degree of renal damage had previously been achieved. Only two groups of workers have regularly produced renal vasoconstriction by the infusion of hemoglobin solutions.

The importance of tubular necrosis from nephrotoxins and of disruption of tubules (tubulorrhexis) from focal cortical ischemia (the ischemuric episode), in the pathogenesis of acute renal failure has been stressed by Oliver, MacDowell, and Tracy (20) in their excellent paper. They have discussed the importance of combinations of these factors and point out that many causes lead to the single entity of acute renal failure. It has been suggested that tubular damage may lead to unselective back diffusion of glomerular filtrate. Such effects from hemoglobinemia or distilled water cannot have been important in our patients for the reasons set out in the discussion on the validity of the clearances and because the changes in renal function were rapidly reversible.

The intravenous injection of hematin into the dog induces changes in renal function closely resembling those observed in our patients (21). In man hematin rapidly combines with serum albumin to form methemalbumin but this does not occur in the dog (22). Methemalbumin was identified in the plasma of our patients by spectrophotometric and electrophoretic methods (23) and was found in the urine of some of them (24). As methemalbumin is regularly found in the plasma of all patients who have elevated plasma hemoglobin levels the arguments used in the discussion of the role of hemoglobin apply also to methemalbumin. There was no evidence that methemalbumin acted as a renal vasoconstrictive agent in our patients.

Amberson, Jennings, and Rhode (5) attributed the development of acute renal failure after the infusion of hemoglobin solutions in two of their subjects, to some unknown soluble vasoconstrictive agent. Such a substance may be liberated during hemolysis but it must be relatively unstable for it appeared to be lost in a few hours at 4° C. during the preparation of our hemoglobin solution and most of those used by other workers. Its presence cannot be denied.

Erythrocyte stroma has been suggested as a major factor in the genesis of reactions to intravascular hemolysis but Maluf (25) failed to demonstrate differences between the effects of freshly lysed red cells and of hemoglobin solutions given to dogs. On the other hand the intravenous infusion of homologous stroma into the rat (26) or mouse (27) is quickly lethal and the infusion of stroma into the dog has been reported to induce cutaneous petechiae (28). Small "foreign bodies" could induce local renal vasospasm and any vasodilator might promptly reverse their effect.

The intravenous injection of Parathormone resulted in a prompt diuresis and renal vasodilatation in every patient to whom it was given. The exact degree of renal hyperemia could not be assessed as there was some "washing-out" of inulin and Diodrast from the oliguric kidneys. These changes occurred regardless of the presence of oliguria and renal vasoconstriction and were usually maintained for 40 to 60 minutes.

The effects of Parathormone were not found to be related to any specific effect of the hormone on the tubular reabsorption of phosphate and could be elicited equally well with an autoclaved preparation. The effects appeared to be of a non-specific protein nature. It is important to consider this renal vasodilator effect in the study of the effects of Parathormone on the renal clearance of phosphate.

The data reported here indicate that some patients receiving intravenous infusions of distilled water, and having intravascular hemolysis from them, develop reversible renal vasoconstriction and oliguria without clinical evidence of systemic cardiovascular changes. Profound oliguria did not indicate that structural changes had occurred in the renal tubule cells and that function could be restored only after regeneration. If this type of renal response was due to intravascular hemolysis, and not to the infusion of distilled water as such, it could be a determining factor in the genesis of acute renal failure. Renal vasoconstriction from hemolysis could be converted to severe renal ischemia by mild to moderate shock induced

by the reactions to mis-matched blood transfusion, *Cl. Welchii* infections, or blackwater fever. This could provide a partial explanation for the irregular incidence of acute renal failure after intravascular hemolysis.

SUMMARY

1. Intravascular hemolysis has been induced in 10 patients by the infusion of distilled water into a small peripheral vein while renal function was studied.

2. Urine flow became directly correlated with renal plasma flow (Diodrast clearance).

3. Profound oliguria and marked renal vasoconstriction developed in seven of the patients but both rapidly disappeared when the infusion of distilled water was discontinued.

4. Oliguria and renal vasoconstriction could be reversed rapidly by the intravenous injection of Parathormone which caused renal hyperemia.

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THE DETERMINATION OF THE SURVIVAL OF TRANSFUSED RED CELLS BY A METHOD OF DIFFERENTIAL HEMOLYSIS [1]

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The technique of differential agglutination of red cells, introduced by Ashby (1), has been widely used to measure the life span of red cells following transfusion. The technique of differential hemolysis was investigated as a possible alternative to this method in an attempt to separate more easily the surviving donor cells from those of the recipient.

Differential hemolysis was first used by Todd and White (2) in studies on the survival of the red cells of one bull when transfused into the circulation of a second bull. Willenegger (3) employed a method of differential hemolysis to study the survival of normal human erythrocytes transfused into the circulation of normal human recipients.

Differential hemolysis is employed in the study of survival of red cells in a manner similar to differential agglutination. Compatible but antigenically distinct red cells are introduced by transfusion into the circulation of a suitable recipient. The survival of these transfused red cells can then be followed during their life span in the recipient by taking samples of blood containing the mixed cell population, eliminating the cells of the recipient by exposing the mixture to the appropriate hemolytic antiserum, and counting the remaining number of donor cells which are not subject to hemolysis. The method of differential hemolysis is different from that of differential agglutination in that hemolysins are used instead of the corresponding agglutinins in the separation of the mixed cell population. Since Anti-A and Anti-B are the only isoantibodies which occur in the form of potent hemolysins the technique is limited to transfusions within the ABO system and, unlike the method of differential agglutination, cannot be used in studies dependent on the M, N, or D antigens.

The ability of the hemolytic sera employed to hemolyze all but a very small fraction of the cells of the appropriate blood group is obviously of importance if the method is to be reliable. It was found that even the most potent hemolytic antisera did not lyse all the cells in any instance, and that a small number of unhemolyzed red cells always remained. In any prospective recipient for a study of cell survival it is necessary, therefore, to determine the number of his cells per mm^3. which are not hemolyzable by the hemolytic antiserum to be used in the study. This number, which corresponds to the unagglutinable or blank count in the method of differential agglutination, will be referred to as the blank count. If the method of differential hemolysis is to be reliable in the estimation of the survival of transfused cells the blank counts of any hemolytic antisera used must conform to the specifications established for satisfactory antisera in the method of differential agglutination. Accordingly, the blank count must be low. About 20,000 cells per mm^3. is usually regarded as the upper limit permitted (4). Also, the blank count should not vary significantly on storage of the serum for periods of at least five months.

To assess the reliability and accuracy of the method of differential hemolysis, the survival of transfused red cells was estimated in parallel by this technique and by a standard method of differential agglutination in seven individuals. Blank counts were performed on a number of normal individuals and on patients in a general medical

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ward. In some cases these counts were repeated after an interval of some months to determine whether or not any significant change occurred. The number of group O cells was also estimated in known mixtures of these with either group A or group B cells.

MATERIALS AND METHODS

Transfused blood

Blood from group O donors was taken into a standard acid citrate dextrose solution [4] and stored at 2° C. In no instance was the duration of such storage prior to transfusion longer than 48 hours. Immediately prior to transfusion in each case the plasma was removed and, after washing the cells once in an equivalent volume of saline, this also was removed and replaced with a further volume of saline. Cross matching was performed in each case in 30 per cent bovine albumin and by means of an indirect Coombs test by standard procedures (5).

Estimation of surviving donor cells

1. *Method of differential agglutination.* Dried immune rabbit Anti-A, Anti-B, and Anti-M sera (Lederle) which gave satisfactorily low blank counts in each case were used in the procedure described by Ebert and Emerson (6) with only minor modification. Dilutions of whole blood were made in two ways. As described by Emerson (7), 0.1 ml. of whole blood was pipetted into 0.9 ml. of 0.85 per cent sodium chloride solution to make a 1 in 10 dilution. One-tenth ml. of this 1/10 dilution was then added to 1.9 ml. of 0.85 per cent sodium chloride solution to produce a final dilution of 1 in 200. A final dilution of whole blood of 1 in 204 was prepared in other instances in the following manner: A sample of 0.1 ml. of whole blood was measured within the accuracy of a Folin and Wu pipette [5] and delivered into 5.00 ml. of 0.85 per cent sodium chloride solution to produce a dilution of 1 in 51. Using an automatic glass pipette (4), which delivered approximately 0.1 ml., one volume of the red cell suspension (1 in 51) was mixed with three volumes of 0.85 per cent sodium chloride solution to produce a final dilution of 1 part of whole blood in 204 parts of sodium chloride solution. In performing blank counts the cells in 0.5 or 1.0 mm^3. were counted and in counts of surviving red cells a total of 500 to 1,000 cells usually was counted on two sides of the same counting chamber. When the number of surviving cells was small, the cells in 0.5 mm^3. on each side of the chamber were counted.

2. *Methods in differential hemolysis.* Immune human Anti-A and Anti-B sera were used. At the commencement of this study immune sera were obtained from a patient of group O who had inadvertently been given 500 ml. of group A blood. Sera from this patient (DB1 and DB2) were obtained 10 and 24 days after this transfusion of incompatible blood and contained an Anti-A hemolysin capable of giving satisfactory blank counts if used undiluted. These antisera, however, proved unsatisfactory if diluted at all and could not therefore be used in the method of differential hemolysis described below in which fresh serum was added as a source of complement. More potent hemolytic antisera were obtained from the Knickerbocker Blood Bank, New York, New York. These were prepared by the immunization of group B and group A volunteers, respectively, by two subcutaneous injections of commercially prepared group A and group B specific substance given 24 hours apart. The serum of the volunteers was harvested 10 days later. All sera were stored in amounts sufficient for one determination (about 0.2 ml.) in sealed glass ampoules at − 15° C.

a. *Differential hemolysis without added fresh serum.* About 1 ml. of freshly drawn venous blood was placed, without anticoagulant, onto a paraffined watch glass. A sample of 0.1 ml. of this blood, measured within the accuracy of a Folin and Wu pipette, was delivered into 5.00 ml. of 0.85 per cent sodium chloride solution to produce a final whole blood dilution of 1 in 51. This red cell suspension was then thoroughly mixed by repeated gentle inversion. Using an automatic glass pipette which delivered approximately 0.1 ml., equal volumes of this red cell suspension and of hemolytic antiserum were pipetted into a culture tube of dimensions 10 × 75 mm. which was stoppered and incubated in a water bath at 37° C. for 15 minutes.

b. *Differential hemolysis with added fresh serum.* The procedure as described above was modified as follows. An equal volume of the recipients fresh serum was added, by means of the same automatic glass pipette, to the suspension of red cells before the addition of the volume of hemolytic anti-serum. Thus the final dilution of the sample of blood was 1 in 153 (1 in 51 × 3) in this method instead of 1 in 102 (1 in 51 × 2) in the method described previously. Incubation was carried out as above for 15 minutes at 37° C.

In all cases the same Folin and Wu 0.1 ml. pipettes were used in making dilutions and the same counting chamber was used in cell counts for both methods.

RESULTS

1. *Blank counts*

The number of unhemolyzed cells per mm^3. of whole blood was determined in 19 individuals using the appropriate group specific antisera. At the commencement of this study no fresh serum was added to the mixture of cells and antiserum. Using this technique eight group A subjects were studied using seven antisera and one group B subject using five antisera. The blank counts obtained were satisfactory since none was more than 26,000

[4] Formula: Citric Acid, 0.48 Gm.; Sodium Citrate, 1.82 Gm.; Dextrose, 1.47 Gm.; Water, to 100 ml.

[5] Calibrated by the manufacturer to contain 0.1 ml. with the volume adjusted to a tolerance of ± 0.00025 ml.

TABLE I

Results of counts of unhemolyzed cells (blank counts) of individuals of group A_1 or group B using antisera containing isohemolysins of the appropriate type and with the addition of fresh normal serum as a source of complement

Blood group	Number of subjects	Number of hemolytic antisera used	Number of blank counts performed	Number of unhemolyzed red cells/mm.³ or the "blank count"	
				Mean	Range
A_1	5	8	20	9,500	1,100–22,200
B	5	5	17	12,200	2,800–16,500

per mm³. Of 17 counts on the group A subjects twelve were less than 10,000 per mm³. Blank counts using this technique and the same antisera were repeated over a period of six months in several of these cases, and little change in their magnitude was found for a period of about five months. At this stage, however, presumably due to deterioration in the complement in this antisera, they failed to produce complete lysis and considerably higher blank counts were obtained. At the same time agglutinated masses of unhemolyzed cells were observed when making these blank counts. Prior to this time the small number of unhemolyzed cells, representing the blank count, had shown no tendency to form agglutinates. To overcome this difficulty complement, in the form of freshly drawn serum from the subject, was added to the saline suspended cells prior to the addition of the hemolytic antiserum. With suitable sera satisfactory blank counts could be obtained by this method even after complete destruction of complement in the antiserum by heating it to 56° C. for 15 minutes. Reconstituted lyophilised guinea pig serum [6] was also tried as a source of complement in similar experiments but proved less satisfactory because of less complete lysis of cells and a tendency for the unlysed cells to form agglutinates. No agglutinates were observed when fresh human serum was used as a source of complement.

Using this method with added fresh serum, blank counts were done on five group A_1 subjects, five group B subjects and four group AB subjects. No group A_2 subjects were studied. Eleven Anti-A sera and nine Anti-B sera were used of which eight Anti-A and five Anti-B sera proved satisfactory since the blank count was less than 30,000 red cells per mm³. The unsatisfactory sera gave blank counts in excess of 30,000 per mm³. for group A and B subjects, although all but two of them appeared to produce complete hemolysis on examination of test cells by the unaided eye. The details of blank counts obtained with the 13 sera with low blank counts are shown in Table I, and the results of blank counts on group AB subjects are shown in Table II.

In several instances the blank counts of subjects were repeated, using the same antisera, after varying intervals of time. These included the recipients used in red cell survival studies where blank counts were done both at the commencement and conclusion of the study. These data are shown in Table III. It can be seen that changes in the blank count are not of sufficient magnitude to interfere significantly with the accuracy of a red cell survival study. Thus a change in blank count from 13,000 per mm³. to 10,000 per mm³. would have little effect on the accuracy in the estimation of several hundred thousand group O cells per mm³.

TABLE II

Results of counts of unhemolyzed cells (blank counts) of four group AB individuals using antisera containing Anti-A or Anti-B isohemolysins and with the addition of fresh normal serum as a source of complement

Subject	Blood group	Hemolytic antiserum	Blank count (cells)* mm.³
J. D.	A_1B	Anti-A (NYA2)	29,000
		Anti-B (NYB1)	333,000
E. R.	A_2B	Anti-A (NYA4)	1,500,000
		Anti-A (NYA5)	1,500,000
		Anti-B (NYB1)	12,200
		Anti-B (NYB5)	12,200
J. K.	A_1B	Anti-A (NYA4)	303,000
		Anti-A (NYA2)	279,000
		Anti-B (NYB1)	44,000
		Anti-B (NYB4)	37,600
V. S.	A_2B	Anti-A (NYA4)	2,760,000
		Anti-B (NYB5)	7,000

[6] Obtained from Carworth Farms, Inc., New City, New York. These guinea pig sera, which were used in final dilutions ranging from 1 in 3 to 1 in 9, were not absorbed with human cells before use.

* As can be seen from the blank counts obtained, either Anti-A or Anti-B sera gave almost complete hemolysis of the cells of any one individual. In all four subjects, however, only one type of antiserum was effective in this way, the antisera of the other type leaving approximately 5 to 45 per cent of the total number of cells unhemolyzed.

TABLE III

Data obtained by repeating counts of unhemolyzed cells (blank counts) on five individuals after varying intervals of time

Case No.	Blood group	Hemolytic antiserum	Interval in weeks	Blank count cells/mm.³
1	B	Anti-B (NYB1)	0	15,000
			23	15,000
			48	16,500
		Anti-B (NYB4)	0	12,400
			23	11,400
			48	13,100
		Anti-B (NYB5)	0	16,500
			23	10,100
2	A_1	Anti-A (NYA2)	0	8,000
			23	14,000
			27	9,000
		Anti-A (NYA4)	0	10,400
			23	10,000
			27	14,600
		Anti-A (NYA5)	0	15,000
			23	12,000
			27	7,100
6	A_1B	Anti-A (NYA2)	0	29,000
			22	12,400
			44	24,000
7	B	Anti-B (NYB5)	0	2,800
			11	7,000
Normal subject	B	Anti-B (NYB1)	0	8,100
			4	13,500
			17	10,000
			44	11,000
		Anti-B (NYB4)	0	12,500
			4	12,900
			17	13,000
			44	13,000
		Anti-B (NYB5)	0	10,000
			4	8,400
			17	9,000
			44	13,000

2. *Counts on known cell mixtures*

Mixtures of known proportions of group O red cells with either group A or group B cells were made. This was done by preparing suspensions of group O and either group A or B cells in saline and determining their cell content by counting 2,000 to 4,000 cells. A microburette was then used to prepare a mixture of the group O cells with either group A or B cells containing a known number of group O cells. The samples were measured promptly to reduce to a minimum the settling of red cells within the burette. The mixtures were so prepared that the number of group O cells was in the range usually encountered in performing cell survival studies. Standard techniques, as described above under methods, were used to estimate the number of group O cells in these mixtures. All sera used in the hemolytic method had been stored for a period of five months and counts were performed in duplicate using two separate samples of each serum. The results are shown in Table IV. The coefficient of variation attributable to random distribution of cells in the chamber in these experiments is estimated to be about 3 per cent since approximately 1,000 cells were counted in each instance.

3. *Red cell survival studies*

The survival of transfused group O red cells was studied in seven individuals using in each instance a method of differential agglutination in parallel with one or both methods of differential hemolysis. A summary of the donors, recipients, methods and sera employed is given in Table V. The results of these survival studies have been plotted in Figure 1. In Cases 1, 2, and 6 the mean of the two counts has been plotted. These were obtained from the use of two separate hemolytic antisera in each observation on the number of surviving donor cells. In Cases 1 and 2 the agreement between duplicate counts was good when using different hemolytic antisera with added complement. Of seventeen such paired observations

TABLE IV

Counts of group O cells in known mixtures with either group A or group B cells as determined by the method of differential hemolysis with added fresh serum as a source of complement

Hemolytic antiserum	Count of group O cells in known mixture: Estimated count, 10^6/mm.³	Count of group O cells in known mixture: Actual count, 10^6/mm.³	Deviation of actual count from estimated count.* per cent
Mixture of group O and group A cells			
Anti-A (NYA2)	0.436	0.437	+0.23
Anti-A (NYA2)	0.436	0.434	−0.46
Anti-A (NYA4)	0.436	0.437	+0.23
Anti-A (NYA4)	0.436	0.443	+1.61
Anti-A (NYA5)	0.436	0.438	+0.46
Anti-A (NYA5)	0.436	0.447	+2.52
Mixture of group O and group B cells			
Anti-B (NYB1)	0.425	0.425	0
Anti-B (NYB1)	0.425	0.418	−1.64
Anti-B (NYB4)	0.425	0.452	+6.35
Anti-B (NYB4)	0.425	0.411	−3.29
Anti-B (NYB5)	0.425	0.427	+0.47
Anti-B (NYB5)	0.425	0.415	−2.35

* The coefficient of variation is about ±3 per cent since approximately 1,000 red cells were counted in each observation.

TABLE V

Details of red cell survival studies shown in Figure 1

Case number	Blood group of recipient	Blood group of donor	Antisera used for differential agglutination	Differential hemolysis: Antiserum used	Differential hemolysis: Added fresh normal serum	Comments
			Group 1—Normal red cells transfused into normal recipients			
1	B M	O N	Anti-M	Anti-B (NYB1) Anti-B (NYB4)	+ +	Normal male, aged 23, was transfused with fresh normal donor blood.
2	A	O	Anti-A	Anti-A (DB1) Anti-A (DB2) Anti-A (NYA2) Anti-A (NYA5)	0 0 + +	Normal male, aged 27, was transfused with fresh normal donor blood.
			Group 2—Normal red cells transfused into patients with anemia			
3	A	O	Anti-A	Anti-A (DB1)	0	Normal donor blood was transfused into a 20-year-old female who was convalescent from an episode of hemolytic anemia associated with moderate thrombocytopenia.
4	A	O	Anti-A	Anti-A (DB1)	0	Normal donor blood was transfused into a 25-year-old female suffering from atypical nonspherocytic hereditary hemolytic anemia. Splenectomy had been performed twenty months prior to this study without benefit.
5	A	O	Anti-A	Anti-A (DB1)	0	Normal donor blood was transfused into a 67-year-old female with refractory macrocytic anemia. Her bone marrow showed a megaloblastic maturation defect but no response had been obtained with usual dosage of vitamin B 12.
			Group 3—Abnormal red cells transfused into normal recipients			
6	A B	O	Anti-A	Anti-A (NYA2) Anti-A (DB1)	+ 0	Blood from a patient with typical congenital methemoglobinamia was transfused into a normal male aged 30.
7	B	O	Anti-B	Anti-B (NYB5)	+	Blood from a subject with elliptocytosis was transfused into a 60-year-old male convalescent from a compound fracture of the leg.

all but three were within twice the calculated standard deviation (Table VI) to be expected from random distribution of cells in the counting chamber. This standard deviation was calculated by taking the square root of the total number of cells counted using both antisera and multiplying by the dilution factor (8).

In Case 2, agreement between these paired observations using the method of differential hemolysis without added complement was not good. In all observations made after the sixtieth day the sera employed *without* added complement were found to be ineffective in hemolyzing all but a small fraction of the recipient's cells which were seen in clumps in the counting chamber when counts of the surviving donor cells were performed. A similar observation was made at about the same time and using one of the same antisera (DB1) in Case 6. In both instances the serum had been stored for a period of about six and one-half

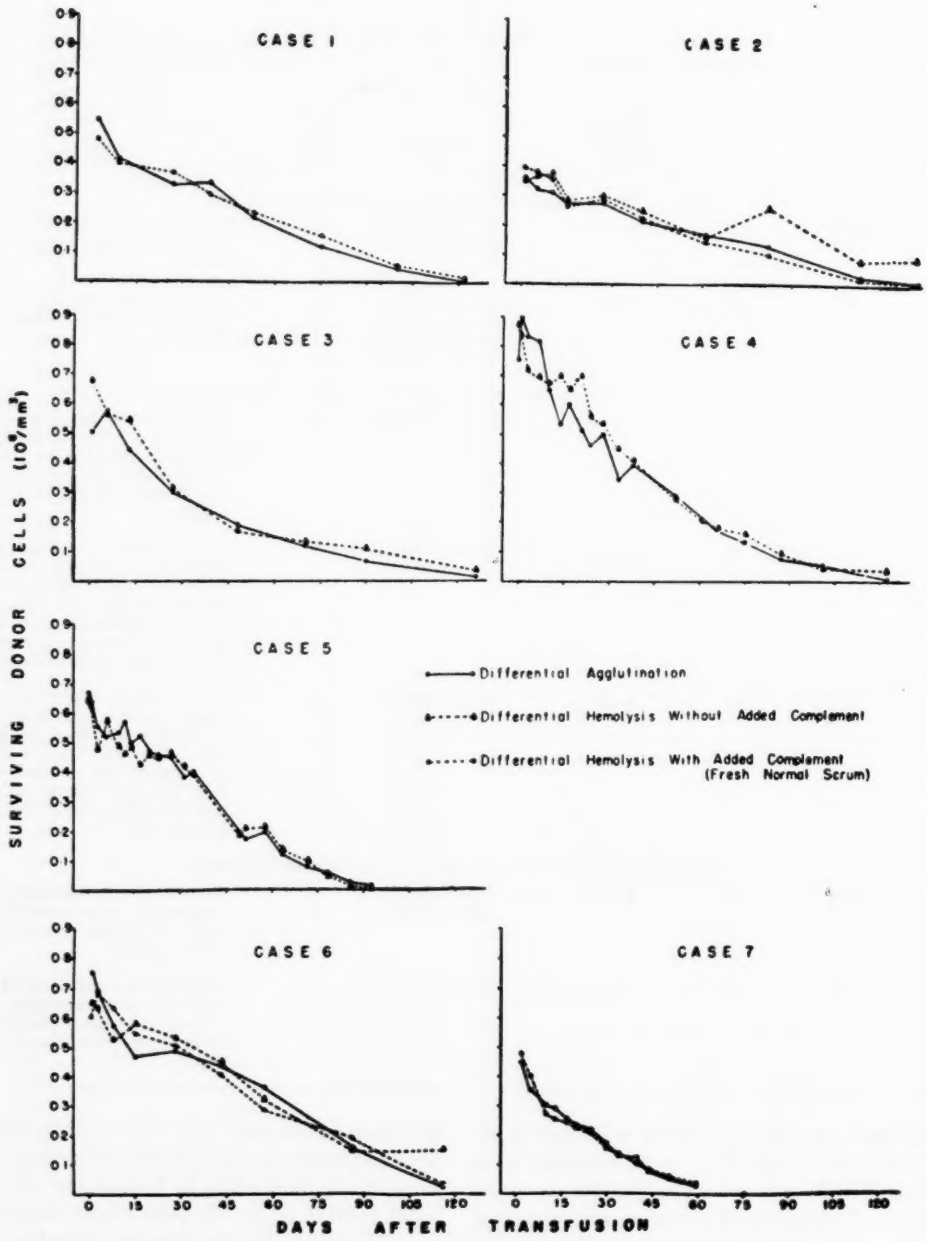

FIG. 1. THE SURVIVAL OF TRANSFUSED GROUP O RED CELLS IN SEVEN INDIVIDUALS

In each case this has been estimated in parallel by the methods of differential agglutination and differential hemolysis. Results are expressed as the number of surviving donor cells present per mm³. of whole blood.

TABLE VI

Data of parallel estimctions of surviving donor cells in Cases 1 and 2 using two different hemolytic antisera in the method of differential hemolysis with the addition of fresh serum as a source of complement

Days after transfusion	Number of surviving donor cells ($10^6/mm.^3$)		Difference between two observations ($10^6/mm.^3$)	Anticipated error (2 S.D.) due to random distribution of cells in counting chamber ($10^6/mm.^3$)
		Case 1		
	Anti-B (NYB1)	Anti-B (NYB4)		
2	0.592	0.500	0.092*	0.058
9	0.400	0.417	0.017	0.041
27	0.340	0.311	0.029	0.032
38	0.333	0.335	0.002	0.033
53	0.222	0.225	0.003	0.027
75	0.118	0.125	0.007	0.013
100	0.060	0.048	0.012*	0.009
122	0.014	0.014	0.000	0.005
		Case 2		
	Anti-A (NYA5)	Anti-A (NYA2)		
1	0.408	0.375	0.033	0.040
5	0.373	0.387	0.014	0.039
10	0.361	0.341	0.020	0.038
15	0.251	0.280	0.029	0.030
27	0.277	0.281	0.004	0.034
40	0.236	0.223	0.013	0.022
61	0.145	0.140	0.005	0.013
82	0.097	0.101	0.004	0.012
113	0.023	0.035	0.012*	0.007

* The difference between the parallel observations in these observations exceeds 2 S.D.

months since its collection. The loss of hemolytic activity was presumably due to a fall in the content of serum complement, but complement titrations were not performed. Also these particular Anti-A sera (DB1 and DB2) did not contain a sufficiently potent isohemolysin to be effective when diluted by the addition of fresh serum in the method of differential hemolysis with added complement. In both Cases 2 and 6 the blank count by the method of differential hemolysis without added complement finally rose to over 100,000 per mm^3. at the conclusion of the studies.

Apart from the unsatisfactory agreement resulting from high blank counts in the method of differential hemolysis without added complement, the agreement between the number of surviving donor cells, as estimated by the methods of differential agglutination and differential hemolysis was good. It is also apparent that there was no systematic error involved since neither method gave consistently higher or lower readings than the other. Agreement between the methods was most satisfactory in Case 7 which was the last to be performed, a fact which may be attributable to increasing experience in the use of both the methods of differential agglutination and differential hemolysis.

DISCUSSION

The results obtained demonstrate the possible applications and limitations of the method of differential hemolysis to estimate the survival of red cells following transfusion. Experience with the method of differential hemolysis in which no complement was added to the system showed that this technique was not reliable if the hemolytic antisera were stored for periods in excess of about five months. This was due to the fact that the blank counts obtainable with such sera rose after this time due presumably to the loss of hemolytic complement activity. This difficulty was overcome for potent hemolytic antisera by the addition of complement in the form of fresh serum to the system.

The method of differential hemolysis with added complement proved to be a practical and reliable method for the determination of red cell survival in the cases studied. Features which were considered satisfactory in the method included the

following: The blank counts with potent antisera were low and compare favorably with those reported for the method of differential agglutination especially for recipients with blood of group B. The method of differential hemolysis obviated the separation of the donor cells from the agglutinated masses of recipient cells by the rather arbitrary mechanical means of centrifugation and agitation which are a feature of most methods of differential agglutination. By eliminating the clumps of agglutinated recipients cells from the counting chamber, the method of differential hemolysis also facilitated the counting of surviving donor cells. For this reason also the technique of differential hemolysis would appear to be more satisfactory for counts done by means of an automatic red cell counter.

The method of differential hemolysis permits easy separation, with a minimum of manipulation, of a population of donor's cells with a decreasing life expectancy in the circulation of the recipient. Such a technique might be used to study more closely the changes occurring with the process of aging of the red cell *in vivo*.

The method of differential hemolysis is limited in scope since red cells with M and N antigens cannot be studied because the antibodies to these antigens are not hemolytic.

Several observations were made in connection with the action of group specific isohemolysins. The resistance of a very small fraction of the cells to hemolysins by even the most potent antisera as represented by the blank count, is analogous to the unagglutinated red cells observed with the most potent isohemagglutinins. It is difficult, however, to explain the observation of unhemolyzed cells by means of the "saturation hypothesis" of McKerns which is the presumed mechanism of the unagglutinated cells. McKerns and Denstedt (9) postulate that unagglutinable cells become saturated with antibody before they have a chance to collide with other cells and that the complete saturation of their antigen receptors leaves none of these available for reaction with antibody fixed to other free or agglutinated cells. Another possible explanation for lack of hemolysis would be the consumption of hemolytic antibody or of complement. This, however, was evidently not the case since red cells added to a system containing this small number of unhemolyzed cells were readily hemolyzed showing that neither antibody nor complement had been exhausted. The mechanism responsible for unhemolyzed cells is not known.

The observation that fresh normal human serum proved a more satisfactory source of hemolytic complement than reconstituted lyophilised guinea pig serum in conjunction with the action of isohemolysins on the cells of the appropriate blood group was unexpected. In other reactions involving complement, guinea pig serum is found to be more effective than normal human serum because of the higher complement titre of the former.

Finally, the observation that the great majority of the red cells of a group AB individual were susceptible to hemolysis by either Anti-A serum or Anti-B serum but not by both was interesting. The fact that 5 to 45 per cent of all the cells were not hemolyzed by one type of antiserum suggests an unequal distribution of either the A or B antigen on the cells of these group AB subjects. However, more evidence is needed to support this hypothesis.

The survival of normal red cells transfused into normal recipients showed a life span of these cells of about 120 days—a result which is in agreement with previous similar studies. The normal life span of the red cells from a patient with congenital methemoglobinemia and the decreased life span of elliptocytes in the circulations of normal recipients are also of some interest.

SUMMARY

1. A reliable technique for the estimation of the life span of transfused group O cells is described employing a method of differential hemolysis.

2. If potent immune human antisera were used and fresh serum added as a source of complement, blank counts obtained with this technique compared very favorably with those obtained using the technique of differential agglutination.

3. Using this technique with added fresh serum blank counts in individuals did not vary significantly with storage of the antisera at − 15° C. for periods up to 27 weeks.

4. Counts of group O red cells in mixtures of known proportion with red cells of either group

A or group B demonstrated the accuracy and reliability of the technique in the separation of such mixed red cell populations.

5. The survival of transfused red cells in seven recipients was estimated in parallel by techniques of differential agglutination and differential hemolysis. Satisfactory agreement between the two methods was obtained provided fresh serum was added to the mixture of cells and hemolytic antiserum to insure the presence of adequate amounts of complement in the mixture.

6. The advantages and possible application of the technique to the closer study of the changes occurring in the *in vivo* aging of red cells is discussed.

7. Some observations have been made on the actions of isohemolysins.

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MODIFICATION BY ASCORBIC ACID AND BRITISH ANTI-LEWISITE OF THE EFFECT OF MERCUHYDRIN ON RENAL TUBULAR FUNCTION IN MAN [1]

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(Submitted for publication December 23, 1952; accepted February 18, 1954)

Alteration of renal hemodynamics and tubular function following administration of organic mercurial compounds has been investigated by several workers (1, 2). These compounds have no effect on the glomerular filtration rate or effective renal plasma flow. The maximal capacity of the tubules to excrete para-aminohippurate in man (Tm_{PAH}) is depressed by the administration of mercurial diuretics. Berliner, Kennedy, and Hilton (1) observed depression of Tm_{PAH} varying from 67 to 83 per cent of the control values after administration of Salyrgan [2] to five human subjects. Our unpublished observations confirm that administration of Mercuhydrin [3] also depresses Tm_{PAH} to a similar degree. Berliner, Kennedy, and Hilton also found that the depressed tubular excretion of PAH following administration of Salyrgan may be restored to the control value by injection of British Anti-Lewisite (BAL, 2, 3-dimercapto propanol). Ruskin, Ruskin, and Nowinski (3) report that ascorbic acid protects against the respiratory depression of rat kidney slices observed following exposure to Mercuhydrin. This observation suggested to us that ascorbic acid might also prevent the depression of Tm_{PAH} induced by Mercuhydrin. Our experiments demonstrate that ascorbic acid is virtually as effective as BAL in inhibiting the depression of Tm_{PAH} that follows administration of Mercuhydrin.

METHODS

Patients were selected on the wards at random. Those having far advanced renal disease and congestive heart failure were excluded. After fasting overnight the subject was given 1,500 cc. of tap water 30 minutes before each procedure to induce adequate urinary flow. Priming doses of 50 cc. of 25 per cent mannitol and 50 cc. of 20 per cent sodium para-aminohippurate (PAH) were injected intravenously. Continuous intravenous infusions of mannitol and PAH were maintained throughout the course of each experiment so that plasma levels of these substances remained fairly constant. After an equilibration period of 20 minutes, clearances were measured in two successive periods of 15 minutes each and averaged. Urine specimens were collected through an indwelling catheter, and the bladder was irrigated with 20 cc. saline and flushed with air at the beginning and end of each period. Venous blood samples were drawn at the beginning and end of each period. Plasma levels of PAH were maintained above 30 mg. per 100 cc. in all determinations of Tm. Blood and urinary specimens were analyzed for mannitol and PAH by methods described in the literature (4). Clearance of mannitol was regarded as a close approximation of the glomerular filtration rate.

In six subjects, after the control clearance was determined, 150 mg. of ascorbic acid was given intravenously. Then two measurements of mannitol clearance and Tm_{PAH} in successive 15-minute periods were made and averaged. Next, Mercuhydrin, 2.0 cc., was injected and the mannitol clearance and Tm_{PAH} again measured in two periods beginning 30 minutes after the injection. In five other subjects, BAL, 3.0 mg. per kilogram of body weight, was injected intramuscularly at the same time that Mercuhydrin was injected intravenously. Mannitol clearance and Tm_{PAH} were determined in two successive periods beginning 30 minutes later.

RESULTS

Ascorbic acid alone had no significant influence on Tm_{PAH} (Table I). The average Tm_{PAH} in six subjects prior to injection of ascorbic acid was 58.8 mg. per minute compared to 60.8 mg. following ascorbic acid. Ascorbic acid protects against the depression of Tm_{PAH} induced by Mercuhydrin. The average Tm following ascorbic acid and Mercuhydrin was 57.1 per 100 cc., or 94 per cent of the control value. No correlation is apparent between the degree of depression of Tm_{PAH} and the diuretic response.

[1] Supported in part by a Cardiovascular Training Grant from the National Heart Institute, U. S. Public Health Service.

[2] Salyrgan (sodium salt of 0-(3-hydroxymercuri-2-methoxypropyl) carbamyl phenoxyacetic acid) is manufactured by Winthrop-Stearns, Inc., Rensselaer, New York.

[3] Mercuhydrin (sodium salt of methoxy-oxymercuripropyl succinyl urea with aminophyllin) is manufactured by the Lakeside Laboratories, Milwaukee, Wis.

TABLE I

Glomerular filtration rate, Tm_{PAH}, and urine flow following intravenous ascorbic acid and Mercuhydrin

Patient	Glomerular filtration rate cc./min.				Tm_{PAH} mg./min.				Urine flow cc./min.			
	Control	After As.A.*	After As.A. and Hg	%†	Control	After As.A.	After As.A. and Hg	%	Control	After As.A.	After As.A. and Hg	%
M. K.	71.2	73.0	98.3	135	56.2	59.0	48.4	82	4.43	4.60	5.60	122
W. H.	108.9	119.9	147.4	123	75.8	79.8	73.6	92	5.50	4.90	7.10	145
F. H.‡	82.5	69.4	67.5	97	54.5	57.4	55.1	96	3.90	4.20	13.10	312
A. B.‡	88.7	108.5	122.5	113	64.3	58.8	59.2	101	9.94	9.10	17.88	196
W. S.‡	75.4	63.3	78.4	123	47.3	42.8	42.2	100	4.35	4.83	6.13	127
L. F.	103.8	82.4	105.9	128	54.7	66.7	64.2	96	3.35	4.48	8.75	196
Averages	88.4	86.1	103.3	119	58.8	60.8	57.1	94	5.25	5.35	9.76	182

* As.A. = Ascorbic Acid.

† Per cent $= \frac{\text{As.A.} + \text{Hg}}{\text{After As.A.}} \times 100$.

‡ Values uncorrected for surface area.

The results when BAL was given with the Mercuhydrin were similar to those when ascorbic acid was given (Table II). The average control Tm_{PAH} in five patients was 57.1 mg. per minute; following coincident administration of BAL and Mercuhydrin it was 57.2 mg. per minute, almost precisely 100 per cent of the control.

Although both BAL and ascorbic acid protected against a decrease in Tm_{PAH} following administration of Mercuhydrin, moderate diuretic responses occurred. This may have been due to the xanthine contained in Mercuhydrin and to the osmotic effect of the continuous infusion of mannitol. No conclusions are justified regarding inhibition of diuresis by coincident administration of BAL or ascorbic acid with Mercuhydrin.

COMMENT

The capacity of mercurial diuretics to depress the transport of PAH across the renal tubular membrane in man is in contrast to the effect of this agent in the dog, but corresponds to the effect in the rat (5). The protection of Tm_{PAH} from mercurial effect and the inhibition of mercurial diuresis by BAL are in accord with the known properties of this agent. Benesch and Benesch (6) showed that BAL combines stoichiometrically with Mercuhydrin to produce a compound that is physiologically inactive. Handley and Lavick (7) reported that organic mercurials inactivate the succinic dehydrogenase system in the renal tubules of the rat and dog. The succinic dehydrogenase system contains sulfhydryl groups susceptible to combination with mercury. They, as well as Farah and Maresh (8), suggest that this system may be implicated in salt and water resorption, since its protection by BAL coincides with reduced diuretic effect of mercurials. Fawaz and Fawaz (9) concluded, on the contrary, that this constitutes insufficient evidence to implicate the succinic dehydrogenase system in the resorption of salt and water. They point out that its inacti-

TABLE II

Glomerular filtration rate and Tm_{PAH} following administration of Mercuhydrin and BAL

Patient	Glomerular filtration rate cc./min.			Tm_{PAH} mg./min.		
	Control	Hg and BAL	Per cent of control	Control	Hg and BAL	Per cent of control
Q. G.	94.3	110.8	118	47.4	37.1	78
H. C.	91.9	102.2	111	48.8	46.2	95
F. O.	133.3	117.2	88	87.5	109.4	125
A. P.	94.3	86.7	92	59.2	49.3	83
I. B.	122.0	122.3	100	42.8	44.7	104
Averages	107.2	107.8	100	57.1	57.2	100

vation by the mercurials may be only one of many effects of this agent upon the renal tubules.

The ability of ascorbic acid to prevent the decrease in Tm_{PAH} following administration of Mercuhydrin may be predicted from clinical experience that ascorbic acid neutralizes toxic effects of arsenic and mercury. The practice of administering ascorbic acid with mercurial diuretics to provide protection against cardiac intoxication is well founded (10, 11). Our results indicate that ascorbic acid also blocks at least one effect of Mercuhydrin on the kidney, the ability of Mercuhydrin to depress Tm_{PAH}. In this respect it is effective to the same degree as BAL. Speculation suggests that this blocking effect of ascorbic acid may depend upon its capacity as a reducing agent (12). It may reduce soluble mercuric salts, at the cellular level, to insoluble and perhaps physiologically inactive mercurous salts.

Our data are insufficient to indicate whether prevention of the decrease in Tm_{PAH} following injection of Mercuhydrin influences the diuretic potency of Mercuhydrin. The clinical practice of administering ascorbic acid with Mercuhydrin must be evaluated in the light of this possibility. Although Shaffer (13) reported that ascorbic acid potentiates the diuretic effect of Mercupurin, the effect was observed in subjects that had been saturated with ascorbic acid. His observation might not be applicable in the routine clinical use of the two agents.

SUMMARY

Following control determination of glomerular filtration rate and maximal tubular excretion of para-aminohippurate (Tm_{PAH}), these functions were observed in man in two sets of circumstances: (1) Immediately following the intravenous injection of ascorbic acid, and in the same subjects successively, 30 minutes after the intravenous injection of Mercuhydrin; and (2) 30 minutes after the concomitant injections of BAL intramuscularly and Mercuhydrin intravenously. Ascorbic acid alone did not influence glomerular filtration rate or Tm_{PAH}, but protected against the depression of Tm_{PAH} usually induced by Mercuhydrin. Concomitant administration of BAL also protected against the depression of Tm_{PAH} induced by Mercuhydrin. The data do not establish whether diuretic potency of Mercuhydrin is altered by concomitant administration of ascorbic acid. This possibility is suggested, however, and is worthy of further investigation.

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"ESSENTIAL" AND "NONESSENTIAL" AMINO ACIDS IN THE URINE OF SEVERELY BURNED PATIENTS[1]

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(Submitted for publication June 11, 1953; accepted February 10, 1954)

The negative nitrogen balance of thermal trauma is a well recognized phenomenon (1, 2). The meaning and significance of this to body metabolism are poorly understood. Partition of urinary nitrogen may assist in interpreting the significance of nitrogen loss. Walker (3) and Taylor, Levenson, Davidson, and Adams (4) found an increase in nonprotein urinary nitrogen of burned patients. This was chiefly due to undetermined nitrogen, the latter defined as the difference between the total nonprotein nitrogen and the sum of urea, uric acid, creatinine, and alpha-amino nitrogen. To analyze this partition further we have studied the urinary amino acid excretion of severely burned patients. Amino-aciduria has been found after severe thermal trauma. A similar amino-aciduria has been observed after comparable surgical trauma, suggesting that the phenomenon is nonspecific. Similar studies on patients with hypercorticism (Cushing's disease) suggest that the adrenal gland may be operative in the amino-aciduria of thermal trauma.

MATERIALS AND METHODS

The paper chromatographic technic described by Martin and Synge (5), Consden, Gordon, and Martin (6), and Dent (7) was utilized to study the urinary excretion of free amino acids. Hydrolysis of urine was not carried out, and so the role of peptiduria cannot be evaluated.

Aliquots of 24-hour urine collections were analyzed for nitrogen (Kjeldahl) and volumes corresponding to 250 micrograms of total nitrogen were chromatographed two-dimensionally. The first solvent consisted of phenol and water; and the second, of butanol, propionic acid, and water (8). The papers were developed by spraying lightly with 0.1 per cent ninhydrin in ethanol.

Using this technic, urine of normal people is found to contain mainly glycine and alanine with occasional traces of other "nonessential" amino acids. In general, only the lower molecular weight, "nonessential" amino acids are found in urine (9–11).

Five members of our laboratory personnel served as "normals." Figure 1 represents the amino acid pattern of the person in this group showing the greatest excretion of amino acids. It also illustrates our charting conventions. The amino acids have been arbitrarily classified as "essential" and "nonessential" in terms of the latest available information regarding the necessity of their presence in the diet of normal adult human beings in order to maintain positive nitrogen balance (12). Within both groups the amino acids have been arranged in order of increasing molecular weight.

The severely burned

Six severely burned patients were studied. All were brought to the hospital shortly after injury except Case No. 52–51, who was transferred from another hospital 48 hours after injury. All had suffered extensive thermal trauma involving from 15 to 65 per cent of the total body surface.

All patients were in good general health prior to being burned. Treatment was similar in each instance. Plasma and blood were used for colloid replacement according to a standard formula (13). Penicillin and streptomycin were administered in every case. All the burned areas were treated with occlusive dressings. Administration and calculation of oral intake were supervised by a special dietitian. Twenty-four hour urine samples were collected in bottles containing thymol and toluol by means of an indwelling catheter. Aliquots were used for analyses.

The unburned

Similar observations were done on patients undergoing conventional surgical procedures. In order of increasing magnitude these procedures were: excision of fissure in ano, hemorrhoidectomy, herniorrhaphy, total hysterectomy, and subtotal gastrectomy. All operations were elective and all patients in good general and nutritional condition. Gas-oxygen-ether anesthesia was used in all but the first two cases, where spinal anesthesia was utilized. Postoperatively, saline, glucose, and water were administered intravenously until the patient's oral intake was adequate.

In an attempt to evaluate the role of the adrenal glands, three patients with hypercorticism due to fullblown Cushing's disease were studied. Twenty-four hour urine collections were carried out before and after radical subtotal adrenalectomy. A minimum of fourteen days was permitted to elapse after surgery so that any effects due to

[1] These studies were aided by financial support from Departments of the Army and Navy, NR *114–198*.

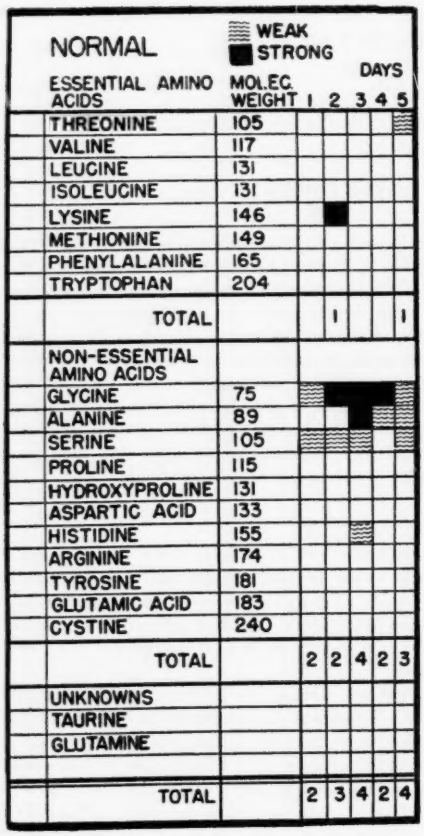

FIGURE 1

surgical trauma *per se* (as seen in the previous group) might be minimized.

RESULTS

The burned

The findings in the burned patients are reported in order of increasing severity. There are two charts for some patients. The first represents the amino acid excretion patterned after Figure 1; the second corresponding chart, suffixed by the letter A, shows the simultaneous over-all nitrogen balance and gross amino-aciduria for the same patient. Since volumes of urine containing equal amounts of nitrogen were chromatographed daily, the significance of the "A" chart becomes obvious. If the amino-aciduria represents a constant percentage of the nitrogen excretion, then chromatography of urine containing equal amounts of nitrogen should result in equal amounts of total amino acid on the chromatogram.

A 19-month-old boy with deep second degree burns of 17 per cent of his body surface (Figure 2, Case 52–88) showed a pattern of amino acid excretion only slightly greater than normal. If taurine and glutamine were to be excluded, the pattern might be considered at the upper limit of our "normals."

A similar pattern is seen in the amino acid excretion of a 42-year-old man who suffered a thermal burn of 30 per cent of his body surface; about half of this was full thickness (Figure 3, Case 52–70). Amino-aciduria subsided somewhat after the first four weeks.

Figure 4 (Case 52–51) demonstrates the amino acids present in the urine of a 30-year-old lineman who suffered deep, high voltage, electrical burns of the entire right arm and both thighs. The arm was amputated at the shoulder and both legs were radically debrided at the time of admission, 48

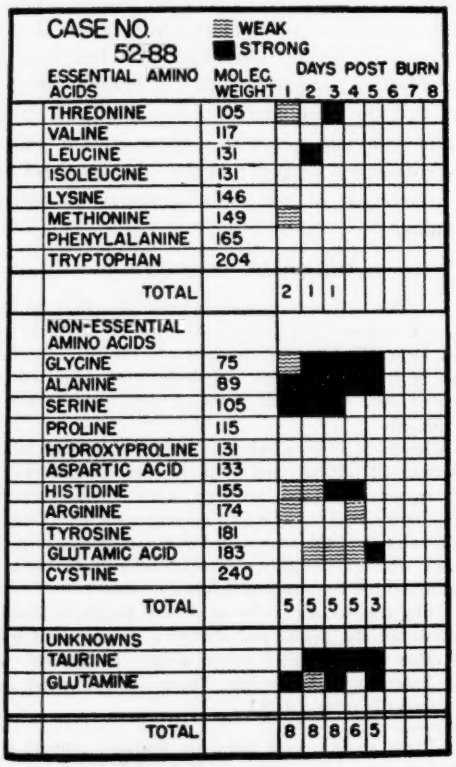

FIGURE 2

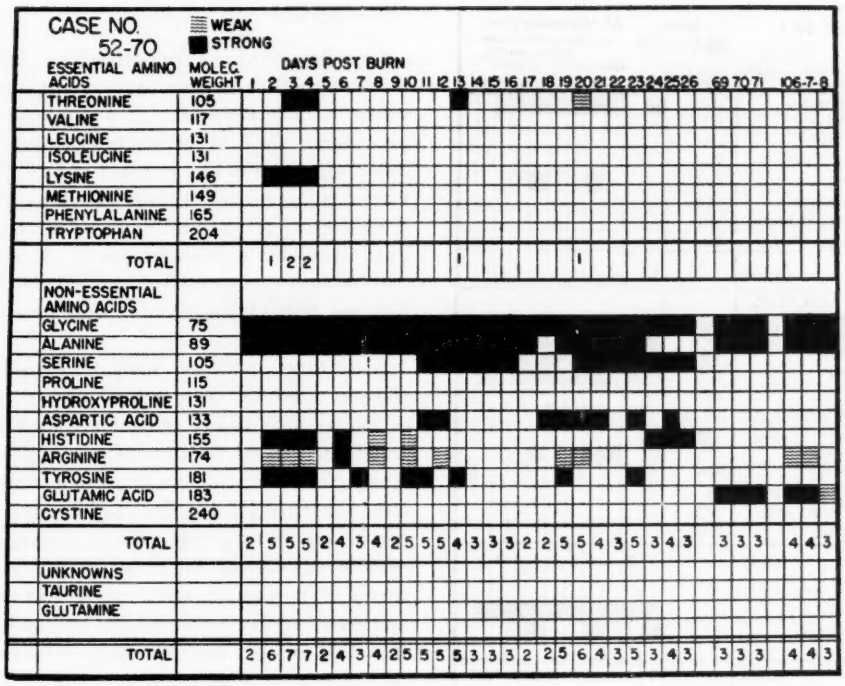

FIGURE 3

hours after injury. His convalescence was uneventful. Taurine and glutamine were again present. The over-all amino acid excretion appeared greater than normal throughout the period of observation. In Figure 4A this patient's nitrogen balance has been charted synchronously with the number of amino acids excreted. Nitrogen balance was calculated by subtracting the grams of nitrogen (Kjeldahl) in the urine, feces, and dressings from grams of nitrogen administered by mouth and parenterally. It is of interest to note the relative constancy of the amino-aciduria despite the shifts in nitrogen balance.

Figure 5 (Case 52–90) represents the amino-aciduria of a 23-year-old man who suffered thermal burns of 40 per cent of his total body surface, most of which were full thickness. Amino-aciduria was still present after forty days, at which time many areas were still unhealed. After 139 days, when grafting had been completed, reduction in amino acid excretion, particularly as regards the "essential" amino acids, is evident.

The amino acid pattern of a 45-year-old woman with 50 per cent body surface burns, over half of which were full thickness, is summarized in Figure 6 (Case 52–92). She died seven days after burning, while in oliguria. The serum nonprotein nitrogen had risen from a value of 31 mg. per cent at the time of admission to 135 mg. per cent two days before death. Figure 6A demonstrates that the period of amino-aciduria corresponded roughly to the period of greatest nitrogen negativity. However, amino-aciduria persisted after restoration of positive nitrogen balance, suggesting that the quantitative and qualitative excretion of amino acids was independent of the over-all nitrogen balance.

A 64-year-old woman with a thermal burn of 64 per cent of her body surface, more than half of which was full thickness, died on her tenth hospital day. Amino-aciduria persisted, though subsiding, until the time of death (Figure 7, Case 52–83). In Figure 7A the amino acid excretion again fluctuates independently of over-all nitrogen balance. The terminal reduction of amino acid excretion may have been due to a reduced glomerular filtration.

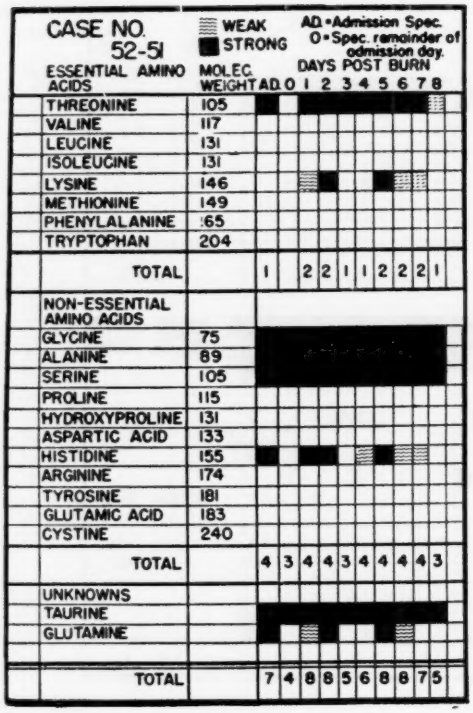

FIGURE 4

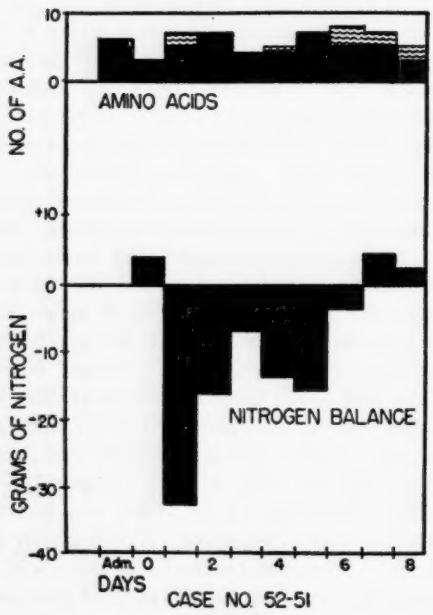

FIGURE 4A

The unburned

Chromatography of the preoperative urine specimens of the five patients undergoing surgery revealed a normal pattern in every case. Minor procedures such as hemorrhoidectomy and excision of fissure in ano resulted in little if any deviation from normal. Partial gastrectomy (Figure 8, Case S–3) resulted in somewhat less amino-aciduria than that seen in Figure 5. Hysterectomy and herniorrhaphy resulted in a pattern which lay between that of gastrectomy and hemorrhoidectomy.

The three patients with Cushing's disease showed a greater than normal urinary excretion of amino acids before operation. Figure 9 illustrates the typical findings in one of these cases. The patient was a 42-year-old woman with a ruddy face, abnormal fat distribution, hirsutism, and hypertension. A two-stage, radical, subtotal, adrenalectomy was carried out under cyclopropane anesthesia. The postoperative course was uneventful and the dosage of cortisone was gradually reduced from 200 mg. per day to a daily maintenance dose of 30 mg. over a period of two weeks. After this maintenance dose had been established, daily urinary collections were carried out for five days. Despite the possible residual effects of surgery, the

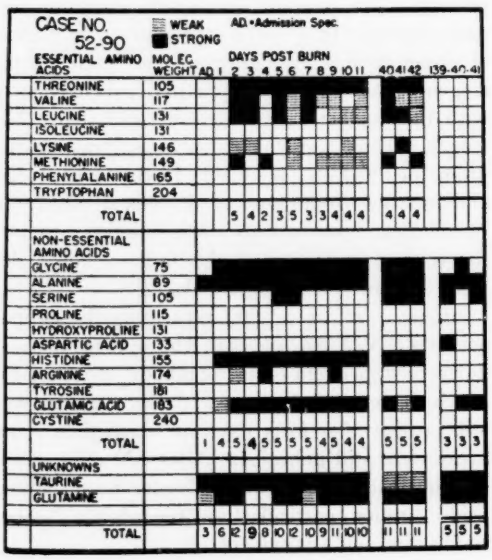

FIGURE 5

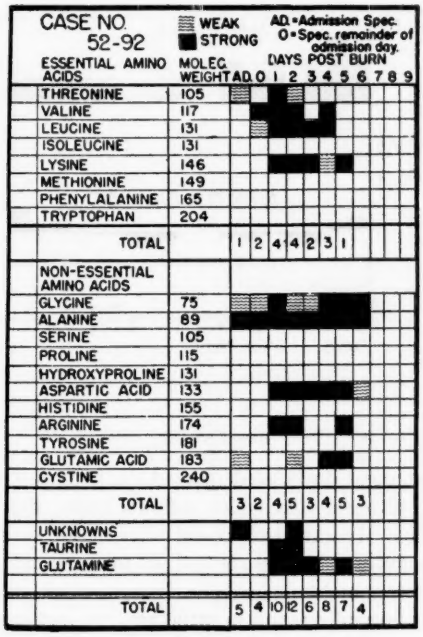

FIGURE 6

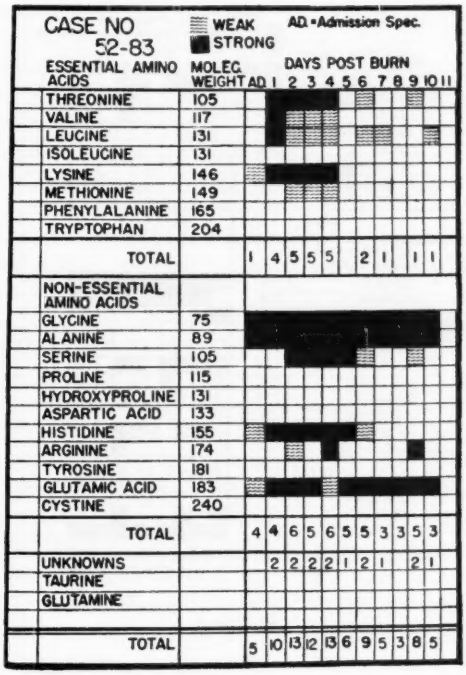

FIGURE 7

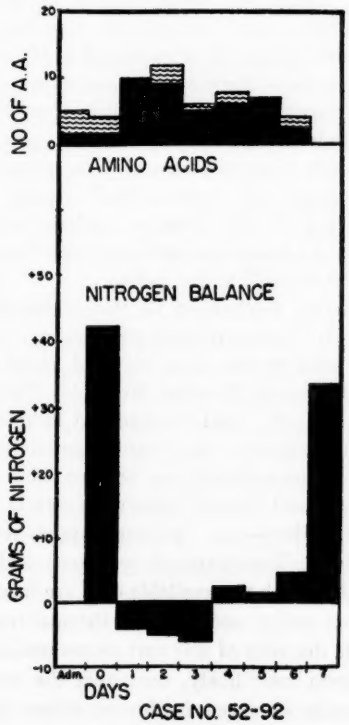

FIGURE 6A

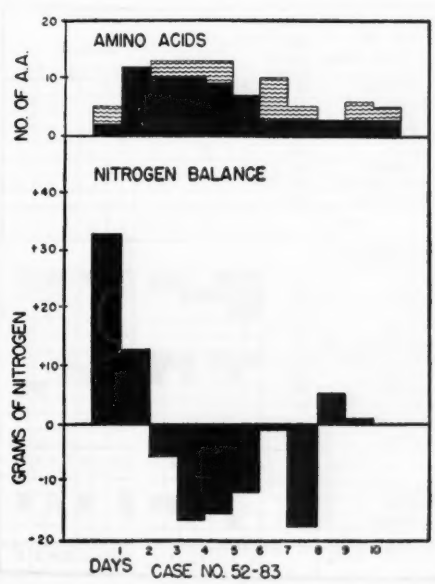

FIGURE 7A

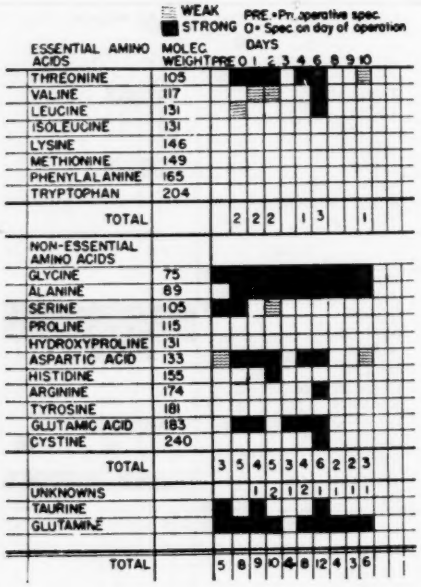

FIGURE 8

minimal amino acid excretion is striking. Figure 9A shows the reduction of amino-aciduria in the postoperative phase as well as the over-all nitrogen metabolism.

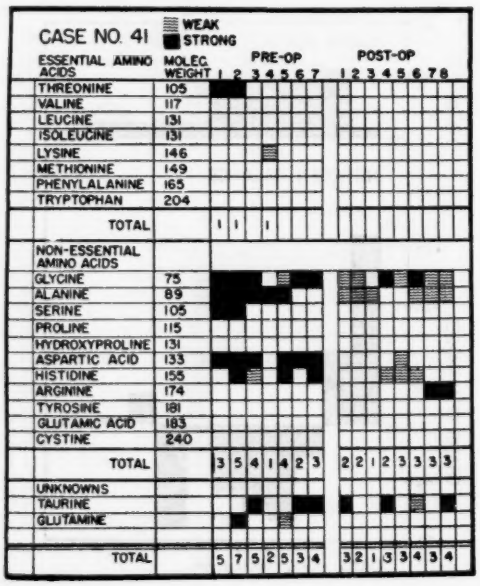

FIGURE 9

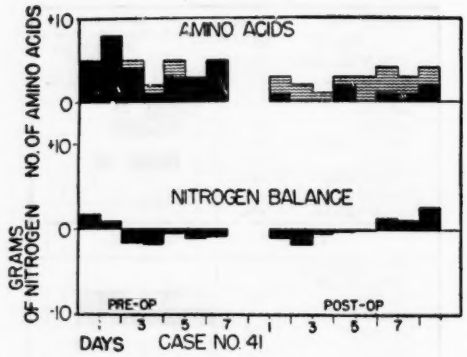

FIGURE 9A

DISCUSSION

Amino-aciduria accompanied the increased urinary nitrogen loss which follows severe thermal trauma. It was also present after major surgical procedures. It consisted of an increase in both the lower molecular weight, "nonessential" amino acids normally found in urine and the higher molecular weight, "essential" amino acids not usually present. There was some suggestion that both the qualitative and quantitative aspects of the amino-aciduria may be proportional to the severity of the trauma. Patients who were not burned severely enough to endanger their lives and patients undergoing minor surgical procedures showed only minimal amino acid excretion with a preponderance of "nonessential" amino acids. Those dying of their burns or undergoing major surgery had a massive excretion of both "essential" and "nonessential" amino acids.

The actual mechanism of this amino-aciduria is not clear. There are three possibilities. Amino-aciduria may result when elevated blood amino acid levels exceed the renal threshold. With normal blood levels, renal damage and failure of tubular reabsorption may cause amino-aciduria. Finally, amino-aciduria may be seen with normal blood levels and "intact" kidneys in certain "metabolic" disorders—*i.e.*, "primary amino-aciduria" as in the de Toni-Fanconi syndrome and cystinuria. Although the available data are inadequate to rule out such a mechanism in thermal trauma, a metabolic disorder of this sort seems unlikely. It would seem most likely, then, that the escape of amino acids observed is due to either elevated plasma amino acid levels or renal dysfunction (al-

though no evidence for the latter has been found), or a combination of these two mechanisms.

Elevation of alpha amino nitrogen in the blood of eight of twelve patients with thermal burns in the first 24 hours after injury has been demonstrated (14). However, marked hemoconcentration was present in these patients. Repeated plasma amino nitrogen levels determined in our patients when hemoconcentration was not present were always found to be within normal limits, *i.e.*, less than 5 mg. per cent.

Everson and Fritschel (15) failed to show any rise in plasma amino nitrogen subsequent to anesthesia and surgery. Friedberg and Greenberg (16) and Li, Geschwind, and Evans (17) suggest that cortical extract or adrenocorticotropic hormone may increase the level of plasma amino acids. Greif (18), however, found a significant fall in the plasma amino acid level of a hypopituitary patient receiving adrenocorticotropic hormone. Russell (19) found that the increase in blood urea in nephrectomized rats resulting from intravenously administered amino acids was markedly reduced by this pituitary hormone.

The high protein intake administered to convalescing burned patients might be a factor in elevating the plasma amino acid level. Dent and Schilling (20) showed that after ingestion of proteins large rises occurred in the concentration of many amino acids in the portal blood of dogs, which could be accompanied by amino-aciduria. It is doubtful that a high protein intake played a significant role in our patients, since the amino-aciduria was subsiding as the patients improved clinically and were able to ingest more protein. In addition, the surgical patients received little or no protein during the first days of convalescence.

Certainly all these factors, protein breakdown, shock, and increased steroid production, are operating in the burned and traumatized patients. However, it seems unlikely that increased blood concentration of amino acids alone can account for the observed amino-aciduria.

After administration of ACTH to a patient, Ronzoni, Roberts, Frankel, and Ramasarma (21) found an increase in urinary amino acid excretion, which returned to normal upon cessation of therapy. However, Russell and Wilhelmi (22) demonstrated an improved rate of de-amination in adrenalectomized rats when cortisone was administered. Burnett (23) suggested that steroid administration improved tubular reabsorption in Addison's disease. Cagan, Klein, and Loewe (24) felt that cortisone caused increased tubular reabsorption of intravenously administered amino acids in treated Addisonians. It is possible that one of the adrenal steroids other than cortisone is responsible for failure of tubular reabsorption, but we have no evidence for this at present. Since adrenal hyperfunction is probably present in all our burns, cortisone may be suspected as a causative factor.

The kidney may play a specific role in the amino-aciduria of trauma. Amino acids seem to resemble glucose in their renal behavior. However, the mechanism of tubular reabsorption must differ since phlorizin poisoning does not produce amino-aciduria. Since amino acids filter freely through the glomerulus, amino-aciduria may result from failure of tubular reabsorption. Pitts (25) has shown a competitive tubular reabsorption for creatin and glycine. Since numerous investigators (26 – 28) have shown the presence of creatinuria in burns, a possible explanation for an accompanying amino-aciduria is suggested. However, since such competition exists only at very high plasma levels, this factor is probably of no importance.

Finally, the influence of amino acids themselves on the renal tubules must be considered. Kamin and Handler (29) have shown that the excretion of threonine and histidine was easily effected by the presence of an unusual quantity of any other amino acid in the plasma. Perhaps the presence of a disorderly, albeit not elevated, pattern of amino acids in the renal tubule is enough to initiate a disturbance of tubular reabsorption.

SUMMARY

An abnormal amino-aciduria consisting of excretion of "essential" amino acids not normally present in urine as well as increased quantities of "nonessential" amino acids was found in the urines of severely burned or traumatized patients.

The qualitative and quantitative aspects of this amino-aciduria may be proportional to the severity of the trauma. The amino acid excretion was influenced by but not completely dependent upon the total urinary nitrogen. Hyperactivity of the adrenal cortex as seen in Cushing's disease resulted in a moderate degree of amino-aciduria. The latter effect was not reproduced by a daily

maintenance dose of 30 mg. cortisone after bilateral subtotal adrenalectomy.

This escape of amino acids is most likely due to a failure of tubular reabsorption. The reason for the latter is not clear but may be due to some adrenal steroid other than cortisone, to an abnormal amino acid pattern presented to the tubule, or to some other factor.

ACKNOWLEDGMENT

The author desires to express his thanks to Dr. Oliver Cope for help and encouragement and to Miss Anne Poulos for invaluable aid in making and interpreting the chromatograms.

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PAPER ELECTROPHORESIS OF SERUM PROTEINS: PHOTOMETRIC QUANTITATION AND COMPARISON WITH FREE ELECTROPHORESIS [1]

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Methods for electrophoretic separation of amino acids, peptides and proteins on filter paper were developed independently by several investigators (1–5) during the years 1948–50. The major protein components of serum could be separated satisfactorily by this principle. The serum proteins on the paper were stained with dye; by cutting the paper into segments and eluting the dye, the amount bound in each segment could be estimated and an electrophoretic pattern constructed. The quicker procedure of photometric quantitation of the resolved serum components directly from the paper strip was introduced by Grassmann, Hannig, and Knedel (6, 7). Variables which affect electrophoretic movement of protein and peptide in a paper supported buffer medium include variations in potential gradient, temperature, pH, ionic strength and nature of the buffer used, thickness of the filter paper, evaporation, time of run, and nature of the protein itself. Paper electrophoresis is well established as a research technique, having been successfully used in serum protein and lipid analysis (2–20), identification of the iron-binding globulin (21), study of gastric mucins (22), serum transport of thyroglobulin (23) and identification of abnormal hemoglobins (24, 25). This report relates experiences with the Grassmann-Hannig and Durrum methods of paper electrophoresis of serum proteins and photometric measurement of the amount of dye bound by the serum protein fractions. The significance of results obtained, their relationship to those obtained by free electrophoresis, and the accuracy and value of paper electrophoresis as a clinical laboratory procedure are discussed.

MATERIAL AND METHODS

Collection of samples. Sera were obtained from fasting subjects and stored at 4° C. Electrophoresis was usually performed on the following day, and not longer than seven days after obtaining the specimen. The results of analyses on 55 subjects are presented.[4]

Paper electrophoresis apparatus and technique. The apparatus described by Grassmann and Hannig (7), and a minor modification of that described by Durrum (26) were used. Both employ similar principles.

Each Grassmann-Hannig box holds two papers in a horizontal position, and three boxes were usually run simultaneously. Strips of Whatman No. 1 filter paper, 4 cm. × 30 cm. were used. After the paper strip had been moistened in buffer, gently blotted and placed in position in the box,[5] approximately 0.01 ml. of serum was carefully applied [6] as a narrow band across the center of the strip to within 0.5 cm. of either edge. Even distribution and linear application of protein were important in determining the quality of the resolution. A diethylbarbituric acid-sodium barbital buffer, pH 8.6, ionic strength 0.1 was used. Several runs could be made with one lot of buffer without change in pH, but the polarity was changed after each run; after every second run, the electrodes were washed free of deposited barbiturate. A power supply and voltage regulator were used to supply constant d.c. voltage from a 110 a.c. volt supply. For six paper strips connected in parallel (three boxes), optimal separations were obtained with 150 v. and 9 m.a. for 14 hours. Although separation could be achieved in 6 to 7 hours with currents of 15 m.a., longer runs with lower currents gave better results. During the run, there was a slight drop in voltage and rise in amperage, due to the properties of the power supply used. No gross fluctuations in cur-

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[4] Samples of abnormal sera were obtained from patients in the King County and Veterans Administration hospitals, Seattle.

[5] The paper was kept under tension by use of counterweights.

[6] "Misco" micro pipettes from the Microchemical Specialties Corp., Berkeley, California.

rent strength occurred, and papers could thus be run conveniently overnight.

A similar technique was employed with the Durrum apparatus. The model used in this laboratory has two buffer compartments 34 cm. by 7 cm. into which the paper dips, and two electrode compartments 34 cm. by 2 cm.; each inner buffer compartment is connected to its corresponding outer electrode compartment by three small openings plugged with glass wool. Up to seven strips of paper, 35 cm. by 4 cm., are supported 20 degrees from the vertical by a plastic rack which has an apex height of 18.5 cm. A diethylbarbituric acid-sodium barbital buffer of pH 8.6, ionic strength 0.1 was employed, with a current of 1.5 m.a. per strip (180–220 volts) for 14 hours. Initially, the dry papers were suspended over the rack and buffer was allowed to gradually soak upwards from the buffer chamber; the serum was applied as a band across the dry section of the paper just before the buffer rising up the two halves of the paper met at the top. It was later found more satisfactory to moisten and blot the entire paper, suspend it horizontally, apply the serum across the center, and then suspend the paper strip over the rack. Movement of albumin during electrophoresis could be followed by adding a small amount of dye (azocarmine or bromphenol blue) to the serum specimen to be used.

Electrophoresis was carried out routinely in the refrigerator at 4° C., although temperature control may not actually be important (7, 27). No studies on the effect of temperature on quality of separation were made in this laboratory.

With either apparatus, best results were obtained with a diethylbarbituric acid-sodium barbital buffer pH 8.6, ionic strength 0.1. Other types of buffers appraised included sodium barbital-acetate, pH 7–9, sodium barbital-citrate pH 8.6, phosphate pH 7–9, phosphate-citrate pH 6–8, and phosphate-citrate-borate pH 7–9, all of ionic strength 0.1. Phosphate buffers are prepared in a simpler fashion and less expensively than barbiturate buffers, permit more rapid movement of serum components, and give a satisfactory separation of β and γ globulins. However, of the buffers evaluated, only barbiturate solutions resolved α_1 globulin from albumin. Protein movement was faster using buffers of ionic strength 0.05, and a run could be completed in 6 to 7 hours; good patterns were obtained but protein "trailing" seemed more pronounced.

After removal from the apparatus, excess moisture was immediately blotted from the buffer-soaked ends of the papers; otherwise this moisture moved towards the center of the strip, displacing the albumin band back towards the point of application and causing some smearing of the pattern. Paper strips were partially dried in air, then transferred to an oven at 100° C. for 30 minutes.

Staining of paper strips. Both amido black 10 B (Grassmann and Hannig [7]) and azocarmine (Turba and Enenkel [4]) stains have been used. Papers were stained for exactly 10 minutes, as it had been emphasized that dye uptake by globulins increases slowly with time (8). For amido black, removal of background stain of the protein-free filter paper required repeated washings

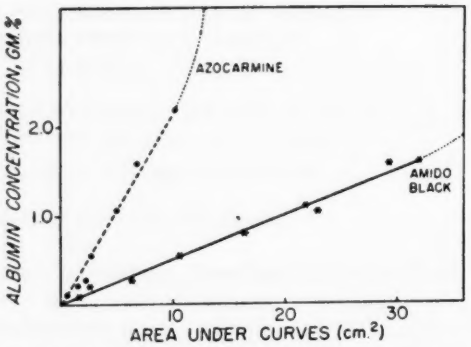

FIG. 1. RELATIONSHIP BETWEEN PLANIMETER AREAS CALCULATED FROM PHOTOMETER READINGS AND QUANTITY OF DYED PROTEIN

with 10 per cent acetic acid in methanol.[7] Decolorization was never quite complete; however the faint blue background did not interfere with photometric registration of protein-bound dye. Azocarmine gave a less intense stain. Prior to its application, protein was "fixed" by immersion of the strip in methanol for 5 minutes. After staining, papers were easily and quickly decolorized with 10 per cent acetic acid. The azocarmine stain faded appreciably in 5 to 6 weeks, whereas amido black did not appear to do so. With either dye, easy decolorization of the protein-free paper with dioxane was not possible, as reported by Griffiths (15) for bromphenol blue. Although azocarmine was easier to handle, and results appeared quantitatively similar to those obtained with amido black, the latter dye was found preferable, particularly for bringing out bands of low protein concentration.

Photometric measure of protein concentration. Papers were rendered translucent by immersion in a solution of α-bromnaphthalene and mineral oil (7) (10 parts of mineral oil to one part of α-bromnaphthalene) before being subjected to photometry.

The Grassmann-Hannig photometer[8] was employed. The oil-soaked paper was passed millimeter by millimeter across a 4 cm. long and 1 mm. wide slit over a white light source, the light absorption being measured by a photocell and registered in optical density units. Errors associated with irregular protein movement at the edges of the paper ("edge effects") were eliminated by shortening the light slit by 1 cm. with black adhesive tape. When consecutive photometer readings (ordinate) were plotted against millimeter distance along the paper strip (abscissa), a smooth electrophoretic curve was obtained showing the characteristic peaks for whole serum components. The percentages of the individual serum protein components present were calculated from planimeter measurements of areas under the peaks, after dropping perpendiculars to

[7] Water washes may be alternated with methanol washes.

[8] Obtained from Bender and Hobein, Munich, Germany.

TABLE I

Results of multiple analyses of a single abnormal serum (Case 52, tuberculous adenitis)

Experiment number	Serum components (per cent total planimeter area)				
	Albumin	Globulins			
		α_1	α_2	β	γ
1	37.0	5.7	14.3	11.9	31.1
2	36.7	5.2	11.9	11.2	35.0
3	39.1	5.7	12.4	11.2	31.6
4	37.5	5.1	13.7	11.0	32.7
5	36.0	5.5	13.2	10.4	34.9
6	35.7	6.4	14.2	11.2	32.5
7	39.2	5.2	13.7	11.6	30.3
8	37.3	6.3	13.4	12.6	30.4
Means	37.3	5.6	13.4	11.4	32.3
Maximum differences	3.5	1.3	2.4	2.2	4.7
Maximum differences from mean	1.8	0.8	1.5	1.2	2.7
Standard deviations from mean	1.20	0.46	0.77	0.61	1.72

the baseline from the lowest points between peaks (28).

Free electrophoretic methods. Free electrophoretic analysis of sera was performed with two different types of apparatus, the Pearson model of the Tiselius apparatus with a slit replacing the knife edge for recording,[9] and the Perkin-Elmer model 38. Sera were diluted 1:4 or 1:4.5 with barbital buffer and dialyzed against buffer for 18 hours at 5° C. Runs were made in a diethylbarbituric acid-sodium barbital buffer, pH 8.6, ionic strength 0.1 at 0–1° C. Magnified schlieren diagrams were traced onto paper and the areas under peaks determined by planimetry. The tracings were divided into areas by lines dropped perpendicularly to the base from the lowest point between the peaks.

[9] Analyses performed by Dr. N. Eriksen.

RESULTS

Readings on the photometer were demonstrated to be directly proportional to amounts of albumin protein on the paper strip, within certain limits of protein concentration (Figure 1): Known quantities of human albumin in 0.01 ml. of solution were applied as a band to the paper, stained, and subjected to photometry. The planimeter areas obtained were then plotted against protein concentration; the relationship of planimeter area to protein concentration was found to be linear only up to 1.6 Gm. per cent using amido black stain, and 2.2 Gm. per cent using azocarmine. These concentrations corresponded, respectively, to protein densities produced by 2.9×10^{-4} and 4.6×10^{-4} mg. albumin per square millimeter averaged over the area scanned by the photometer (1×39 mm.). The density of the albumin band in patterns of normal serum often exceeded these values, being of the order of 9×10^{-4} mg. albumin per square millimeter.

Using amido black, multiple (8) analyses of a single abnormal serum gave a range of variation within satisfactory limits for each component (Table I). The maximum deviation from the mean, in terms of percentage of concentration of total protein, was for albumin 1.8 per cent, α_1 globulin 0.8 per cent, α_2 globulin 1.5 per cent, β globulin 1.2 per cent and γ globulin 2.7 per cent. No difference in electrophoretic separation between the Durrum and Grassmann-Hannig types

TABLE II

Comparison of serum protein staining by amido black and azocarmine dyes

Case No.	Diagnosis	Serum components (per cent total planimeter area)									
		Amido-Black					Azocarmine				
		Albumin	Globulins				Albumin	Globulins			
			α_1	α_2	β	γ		α_1	α_2	β	γ
5	Normal	54.6	4.3	8.9	11.6	20.6	54.6	3.4	9.1	11.3	21.6
10	Normal	60.5	2.4	7.8	13.7	15.6	63.4		7.3	12.7	16.5
18	Portal cirrhosis	44.6	2.7	6.4	10.8	35.5	40.0	4.5	6.9	10.0	38.5
26	Biliary cirrhosis	37.8	13.4		19.5	29.3	36.0	13.6		18.9	31.5
33	Myeloma	36.8	4.2	8.0	9.6	41.4	39.3	5.2	6.8	9.4	39.3
34	Myeloma	24.7	3.4		65.1	6.8	27.8	4.8		62.8	4.6
38	Nephrotic syndrome	38.7	13.3	17.3	15.9	14.8	34.3	11.6	20.1	19.0	15.0
40	Hypercholesterolemic xanthomatosis	60.4	2.7	7.4	14.3	15.1	56.9	2.6	8.3	15.3	16.9
42	Lupus erythematosis	26.7	3.1	8.3	11.0	50.9	24.0	3.5	6.5	10.5	55.5
43	Lupus erythematosis	40.0	7.4	14.1	11.7	24.8	42.4	6.3	13.3	10.5	27.5
45	Myeloid leukemia	45.1	8.2	11.9	9.0	25.8	45.7	7.3	13.7	9.4	23.9
49	Pulmonary abscess	40.4	5.5	11.6	14.2	28.3	40.6	4.9	12.2	15.0	27.3

TABLE III

Comparison of amido black stain and photometric analysis with bromphenol blue staining, elution and colorimetry, and with free electrophoresis

Analytical procedure	Serum components (per cent total planimeter area)				
	Albumin	Globulins			
		α_1	α_2	β	γ
Case No. 9					
Photometric method	55.8	6.0	9.4	13.4	15.4
Elution and colorimetry*	57.4	4.8	9.8	15.1	12.8
Free electrophoresis	58.7	4.8	10.6	12.2	13.7
Case No. 47					
Photometric method	31.1	6.0	7.8	9.0	46.1
Elution and colorimetry*	30.5	3.7	7.6	9.1	49.1
Free electrophoresis	32.4	4.7	6.9	13.8	42.1
Case No. 54					
Photometric method	47.5	5.5	9.5	13.3	24.1
Elution and colorimetry*	47.2	3.5	9.7	15.6	23.8
Free electrophoresis	55.2	4.0	9.6	15.6	15.6

* Data obtained by Drs. B. C. Houghton and C. E. Bender.

of apparatus[10] was observed. Azocarmine and amido black stains yielded almost identical results with a given serum (Table II).

The dye-binding properties of albumin[11] and γ globulin[12] for azocarmine and amido black were studied individually by applying known amounts of protein to paper strips, staining, and measuring by planimeter the areas obtained by photometric measure of dye concentration. When bands representing equal amounts of protein (micro-Kjeldahl nitrogen determination) were compared, the albumin area was found to be greater than that for γ globulin by a factor of 1.3 for amido black and 1.35 for azocarmine.[13] However, this factor did not appear to apply in the following analysis of a normal whole serum: A known quantity of γ globulin was added to the serum of the same subject from which it was obtained. Measurement by both free and paper electrophoresis, employing no factor of correction, almost completely accounted for the γ globulin increment (Table IV). In electrophoretic analysis of whole serum, gamma globulin values were actually slightly greater by the paper method than by free electrophoresis (Table V); this was presumably due to "trailing" of albumin on the paper.

Analyses by paper electrophoresis of 55 different normal and abnormal sera are presented in Table V and Figure 2. In most cases, a corresponding free electrophoretic pattern was obtained, and the

[10] In a limited series, electrophoresis by the above methods, with photometric analysis of the paper strips, gave results which closely corresponded with those obtained by paper electrophoresis between sealed glass plates followed by bromphenol blue staining and elution (Kunkel and Tiselius [5]) (Table III).

[11] Red Cross normal human serum albumin.

[12] Ninety per cent electrophoretically homogeneous normal human γ globulin prepared in this laboratory by Cohn's Method 10 (29).

[13] Bromphenol blue gave erratic results with direct photometry due to instability of color intensity and shade, as was also noted by Kōiw (30).

TABLE IV

Determinations of γ globulin before and after adding known amount of γ globulin to normal serum*

Serum components	Free electrophoresis				Paper electrophoresis			
	Before addition of γ globulin		After addition of γ globulin		Before addition of γ globulin		After addition of γ globulin	
	Per cent of total area	Protein conc. *Gm. %*	Per cent of total area	Protein conc. *Gm. %*	Per cent of total area	Protein conc. *Gm. %*	Per cent of total area	Protein conc. *Gm. %*
Alb	55.7	4.21	47.8	4.23	55.9	4.23	49.1	4.41
α_1	4.7	0.36	4.1	0.36	4.4	0.33	4.4	0.37
α_2	7.6	0.57	7.7	0.68	9.1	0.69	9.3	0.78
β	16.1	1.22	15.2	1.34	11.8	0.89	10.9	0.82
γ	15.9	1.20	25.2	2.23	18.8	1.42	26.3	2.46
Total protein†		7.56		8.84		7.56		8.84
γ globulin increment	2.23 − 1.20 = 1.03 Gm. %				2.46 − 1.42 = 1.04 Gm. %			

* 1.28 Gm., 85 per cent electrophoretically homogeneous (1.09 Gm. γ globulin).
† Biuret.

results for the two techniques were compared. For normal subjects, values for albumin and for the individual globulins fell within the expected ranges, and divergence from free electrophoretic values was small. It is evident from Figure 2 that there is a lack of correspondence in position of the peaks in the paper and free electrophoretic patterns, due to differing protein mobilities between the two techniques. On paper, albumin and α_1 globulin separate well, whereas the other globulins tend to be compressed. This was particularly noticeable in the sera of portal cirrhosis. Without knowing mobilities, precise identification of the various globulin fractions on the paper strip may occasionally be uncertain.

Poor correlation between paper and free electrophoretic analysis was observed in two cases of hypercholesterolemic xanthomatosis tuberosum (Cases 40 and 41). These sera, together with a highly lipemic serum from a patient with the nephrotic syndrome (Case 37), were studied as follows: Total nondialyzable solids (TNDS) were determined by dialyzing the serum against distilled water for 7 to 10 days, then drying the dia-

TABLE V

Comparison of paper and free electrophoresis for normal and pathological sera
(Components expressed as percentages of total planimeter area)

Case No.	Diagnosis	Paper electrophoresis					Free electrophoresis					
			Globulins					Globulins				
		Albumin	α_1	α_2	β	γ	Albumin	α_1	α_2	β	γ	
	Normals											
1		57.8	3.6	8.1	17.8	12.7	60.8	4.0	8.9	15.6	10.7	a
2		65.9	3.9	6.8	12.4	11.0	63.8	5.2	8.1	14.9	7.9	a
3		54.9	5.2	6.9	12.9	20.1	56.7	4.6	7.8	14.8	16.1	a
4		51.5	3.8	9.4	13.8	21.5	57.1	5.3	7.5	14.9	15.2	b
5		54.6	4.3	8.9	11.6	20.6	55.1	4.6	7.5	15.5	16.1	a
6		60.3	4.5	8.9	14.0	12.3	59.2	4.9	8.9	15.1	11.9	a
7		58.7	5.3	9.2	12.1	14.7	54.7	4.7	8.8	18.0	13.8	a
8		57.4	3.6	8.0	14.5	16.5	53.2	4.7	9.0	16.7	16.4	a
9		55.8	6.0	9.4	13.4	15.4	58.7	4.8	10.6	12.2	13.7	b
10		60.5	2.4	7.8	13.7	15.6	56.1	5.9	7.6	14.8	15.6	a
11		59.5	2.4	7.7	15.2	15.2	56.6	5.2	7.6	15.3	15.3	a
12		52.1	4.9	11.2	16.1	15.7	56.6	5.8	10.1	14.3	13.2	a
Means:		57.4	4.2	8.5	14.0	15.9	57.4	5.0	8.5	15.2	13.9	
Mean differences from free electrophoretic values:		−0.	−0.8	−0.	−1.2	+2.0						
Maximum differences from free electrophoresis:		5.6	3.5	1.9	5.9	6.3						
	Portal Cirrhosis											
13		26.4	3.2	6.0	15.3	49.1						
14		36.1	3.1	9.2	15.6	35.9						
15		37.2	4.9	9.9	21.0	27.0						
16		39.6	8.2	15.0	12.8	24.4						
17		40.0	6.8	7.7	13.1	32.4						
18		44.6	2.7	6.4	10.8	35.5						
19		34.6	3.0	9.2	10.0	43.1	34.1	2.9	10.9	11.0	40.9	b
20		38.7	7.2	9.8	11.0	33.3	37.1	5.3	7.8	19.5	30.4	a
21		34.9	4.3	7.6	7.6	45.6	34.0	2.1	5.3	14.5	44.1	a
22		30.6	5.0	8.7	17.2	38.5	28.6	5.2	6.9	17.9	41.4	b
23		26.3	3.2	5.3	9.4	55.8	22.6	3.7	5.6	11.3	56.8	b
24		31.7	5.0	7.3	12.0	44.0	33.7	4.9	6.6	13.8	41.0	b
25		32.4	11.5	10.5	10.4	35.2	33.0	9.1	13.1	10.5	34.3	a
Means:		34.9	5.2	8.7	12.8	38.4	31.9	4.7	8.0	14.1	41.3	
Mean differences from free electrophoretic values (for cases 19–25)		+0.9	+0.9	+0.3	−3.0	+0.9						
Maximum differences from free electrophoretic values (for cases 19–25)		3.7	2.4	2.6	8.4	3.0						

a = Electrophoretic analysis on Perkin-Elmer apparatus model 38 (Ascending limb).
b = Electrophoretic analysis on Pearson model of Tiselius apparatus (Descending limb).

TABLE V—*Continued*

Case No.	Diagnosis	Paper electrophoresis					Free electrophoresis					
			Globulins					Globulins				
		Albumin	α_1	α_2	β	γ	Albumin	α_1	α_2	β	γ	
	Biliary Cirrhosis											
26	Obstructive biliary cirrhosis	36.0	5.4	8.2	18.9	31.5	28.2	5.3	10.0	28.0	28.5	a
27	Obstructive biliary cirrhosis	41.8	6.0	8.2	10.5	33.5	36.6	4.7	8.2	16.3	34.2	a
28	Primary biliary cirrhosis	33.7	7.3	11.6	18.2	29.2	26.4	6.3	9.7	29.9	27.7	b
29	Primary biliary cirrhosis	46.4	4.9	12.2	15.5	21.0	38.0	6.0	12.1	24.1	19.8	a
	Multiple Myeloma											
30		26.9	7.6	11.8	10.0	43.7						
31		46.4	7.7	12.6	16.9	16.4	45.4	9.6	14.9	18.5	11.6	b
32		26.1	2.4	6.4	57.9	7.2	24.4	2.9	7.1	60.9	4.7	b
33		36.8	4.2	8.0	9.6	41.4	26.9	3.5	7.6	7.1	54.8	b
34		24.7	3.4		65.1	6.8	20.0	2.4		75.1	2.5	b
35		28.4	5.8	8.6	7.0	50.2	20.1	5.0	7.0	6.7	61.2	b
	Nephrotic Syndrome											
36	Glomerulonephritis	32.1	6.6	18.9	17.3	25.0	30.6	9.8	20.8	19.3	19.5	a
37	Diabetic nephropathy	37.6	7.4	17.9	22.8	14.3	30.7	7.8	22.6	28.8	10.1	b
38	Diabetic nephropathy	38.7	13.3	17.3	15.9	14.8	30.0	16.3	19.0	18.4	14.3	b
39	Toxemia of pregnancy	22.8	8.3	22.0	21.5	24.5	22.9	15.9	25.8	19.9	15.5	b
	Miscellaneous											
40	Essential hypercholesterolemic xanthomatosis	60.4	2.7	7.4	14.3	15.1	46.0	5.5	10.2	27.7	10.6	b
41	Essential hypercholesterolemic xanthomatosis	53.4	2.3	9.9	16.6	17.8	40.0	4.5	10.5	33.3	11.7	b
42	Lupus erythematosis	26.7	3.1	8.3	11.0	50.9						
43	Lupus erythematosis	42.4	6.3	13.3	10.5	27.5						
44	Lymphosarcoma	57.1	9.1	11.8	11.9	10.1						
45	Myeloid leukemia	45.1	8.2	11.9	9.0	25.8						
46	Neutropenia, recurrent infections	47.1	5.9	11.8	11.4	23.8	50.2	6.2	10.1	14.9	18.6	b
47	Lymphoma	31.1	6.0	7.8	9.0	46.1	32.4	4.7	6.9	13.8	42.1	b
48	Hypopituitarism	24.7	10.9	21.3	12.3	30.8						
49	Pulmonary abscess	40.4	5.5	11.6	14.2	28.3	33.0	6.6	12.2	18.7	29.5	a
50	Recurrent pulmonary infection	51.3	5.8	15.0	11.8	16.1	53.6	7.6	13.5	13.9	11.4	b
51	Staphylococcal infections	53.0	7.5	11.4	12.3	15.8	53.7	6.4	12.1	14.9	12.9	b
52	Tuberculous adenitis	37.0	5.2	14.5	12.7	30.6	36.7	5.1	10.2	14.6	33.4	a
53	Infectious hepatitis	54.9	4.4	6.9	14.1	19.7						
54	Bronchial asthma	47.5	5.5	9.5	13.3	24.1	55.2	4.0	9.6	15.6	15.6	b
55	Agammaglobulinemia	64.4	4.4	10.4	17.8	3.0	66.3	5.5	11.5	14.7	2.0	b

lyzed protein for 72 hours at 85° C., with repeated weighings over this period. The minimum weight was taken as the best estimate of the TNDS of the serum (31). The serum concentration in grams per cent of each component was calculated by multiplying the corresponding per cent of the total planimeter area of the free electrophoretic pattern by the weight of the TNDS.[14] These values were compared with results obtained by paper electrophoresis (amido black) wherein the concentration in grams per cent of each component was determined by multiplying the corresponding per cent of the total planimeter area of the paper electrophoretic pattern by the biuret total protein (Table VI). Values for albumin and γ globulin cor-

[14] The calculation of individual serum components from free electrophoretic patterns as per cent of TNDS would seem more valid than values based upon biuret reaction of the whole serum (32); obviously more substances contribute to refractive change than are determined by dye-staining or biuret-reacting groups, the lipid portions of α and β lipoprotein being good examples.

responded, whereas values for α and β globulins, which contain the lipoproteins, were considerably lower by paper electrophoretic analysis.

In advanced portal cirrhosis, the characteristic albumin depression and γ globulin elevation were consistent findings (Table V). The γ globulin peak of the pattern was usually tall and broad, but certain other abnormalities in this area were also observed. A well-marked and definite band was frequently observed between the β and γ globulin areas corresponding to the previously described γ_1 fraction (33, 34), or H component of Viollier (35). This band was more often apparent in terminal cases, where it occasionally amounted to as much as 12 per cent of the total serum protein

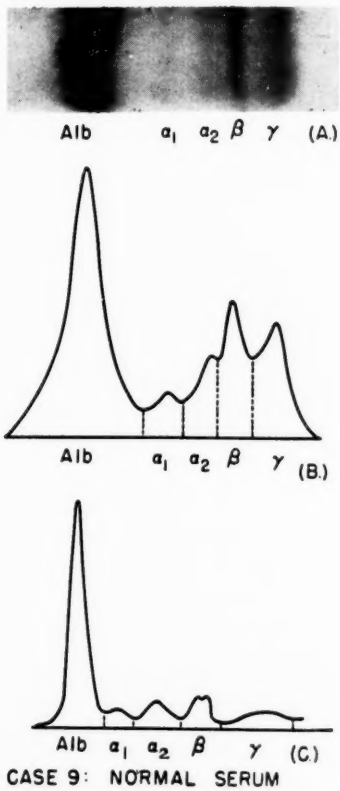

FIG. 2. COMPARISON OF DYED PAPER ELECTROPHORETIC STRIPS AND CORRESPONDING PHOTOMETER PATTERNS WITH TISELIUS SCHLIEREN DIAGRAMS

A = Stained paper strip.
B = Photometer pattern of A.
C = Free electrophoretic pattern.

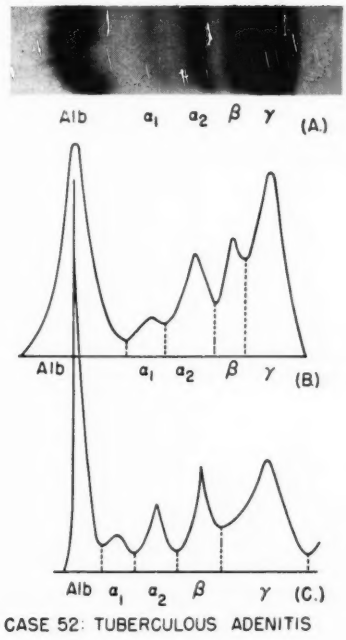
Alb α_1 α_2 β γ (A.)
Alb α_1 α_2 β γ (B.)
Alb α_1 α_2 β γ (C.)
CASE 52: TUBERCULOUS ADENITIS

FIG. 2—*Cont.*

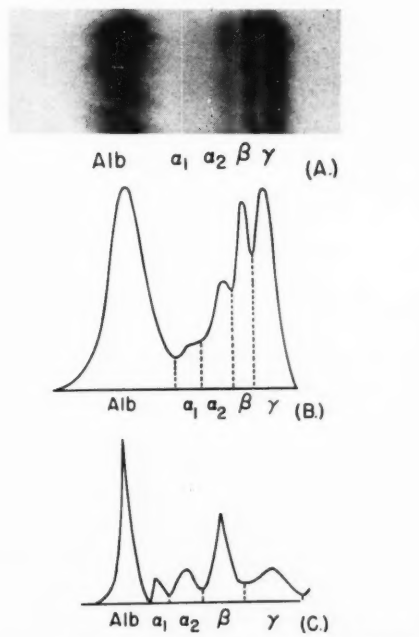
Alb α_1 α_2 β γ (A.)
Alb α_1 α_2 β γ (B.)
Alb α_1 α_2 β γ (C.)
CASE 28 PRIMARY BILIARY CIRRHOSIS

FIG. 2—*Cont.*

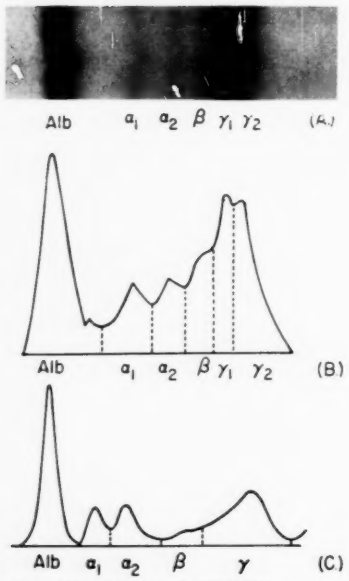
Alb α_1 α_2 β γ_1 γ_2 (A)
Alb α_1 α_2 β γ_1 γ_2 (B)
Alb α_1 α_2 β γ (C)
CASE 25: PORTAL CIRRHOSIS

FIG. 2—*Cont.*

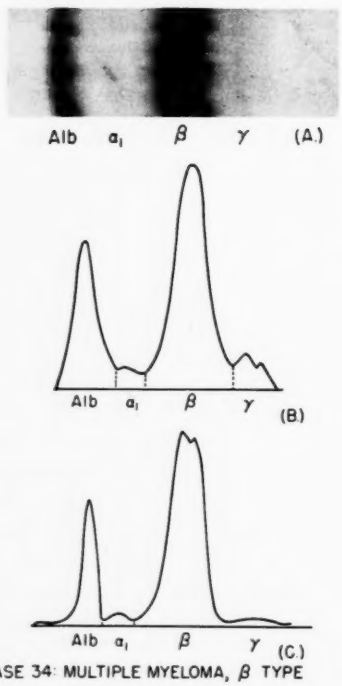

FIG. 2—*Cont.*

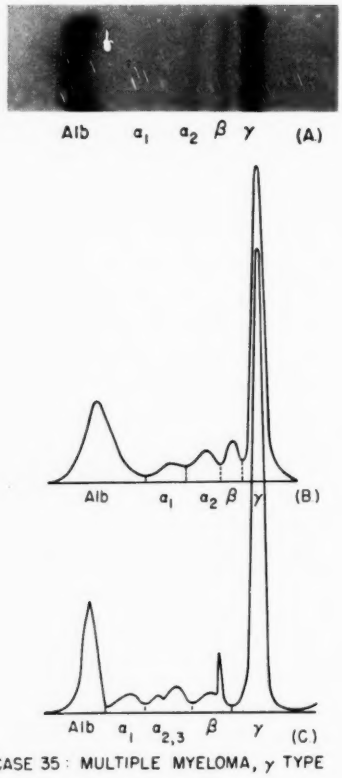
Alb α_1 α_2 β γ (A)
Alb α_1 α_2 β γ (B)
Alb α_1 $\alpha_{2,3}$ β γ (C)
CASE 35: MULTIPLE MYELOMA, γ TYPE

FIG. 2—*Cont.*

concentration, corresponding with Viollier's experience. In our methods of free electrophoresis, this component did not take the form of a discrete peak, but rather produced a plateau effect in the γ globulin area. In other cases of cirrhosis the paper pattern showed lack of clear separation, or smearing together, of the β and γ globulin bands, as noted by Brante (36). Slight α globulin increases were occasionally found, as have previously been noted in cirrhosis (37). Patients with biliary cirrhosis showed similar albumin and γ globulin changes, and also some increase of β globulin, but the discrepancy between the paper and free electrophoretic values for β globulin was marked; the paper method gave values nearly 50 per cent lower than obtained by free electrophoresis.

The seven cases of myeloma are representative of the various protein changes described in this disease (38). Five showed the characteristic narrow spiked peak in the γ globulin area. In Case

26 the abnormal γ globulin was a crystalline cryoglobulin. In Cases 32 and 34, the abnormal protein had a β globulin mobility on free electrophoresis, and accounted for nearly 60 per cent of the total protein present; Case 34 showed a double-spiked β globulin peak. Case 31 was of the unusual normo-proteinemic type. The abnormal myeloma proteins frequently gave lower values on paper electrophoresis as compared with free electrophoretic values. This is believed due to an error in photometric measurement, because the very narrow, deeply-stained myeloma band produced an optical density beyond the linear range of the instrument.

Low albumin and elevated α and β globulins (especially α_2) characterized the sera from the four patients with the nephrotic syndrome. The β globulin values were again found lower by paper than by free electrophoresis. Two cases showed a separate protein component appearing between the albumin and α_1 globulin areas.

In diseased states, measurement of fibrinogen in plasma is unsatisfactory by the paper electrophoretic technique, as has also been found with free electrophoresis (39). Fibrinogen usually appeared as a thin band adjacent to the γ globulin area on the paper, but seldom plotted out as a discrete peak. Particularly in the cases of cirrhosis showing a marked γ_1 globulin band, fibrinogen measurements by electrophoresis would seem impossible.

DISCUSSION

The precision of paper electrophoresis analysis, as evidenced by the reproducibility of results with serum, does not quite equal that of the Tiselius method. For free electrophoresis, Antweiler (40) reports a standard deviation of $\pm$ 0.5 per cent of total protein for each single component, and states that differences between duplicates should not exceed 1.5 per cent of total protein. Not all workers claim this degree of reproducibility (41). With paper electrophoresis, Grassmann and Hannig (7), from multiple determinations on different sera, give standard deviations (as per cent of total protein) of $\pm$ 1.2 per cent for albumin, $\pm$ 0.6 per cent for α_1 globulin, $\pm$ 0.7 per cent for α_2 globulin, $\pm$ 0.7 per cent for β globulin and $\pm$ 1.2 per cent for γ globulin. Sommerfelt (27), with repeated determinations on a single normal serum reports comparable figures. Our reproducibility has also been of this order (Tables I and II); for duplicate analyses, we regard differences within 3 per cent for albumin and γ globulin, and 2 per cent for the other globulin fractions as acceptable. However, when such deviations are related to the concentration in grams per cent of each component, the precision of the analysis is less impressive; large errors in the measurement of the smallest fractions, such as α_1 globulin, by paper electrophoresis are demonstrated.

Protein adsorption on the paper or "trailing," especially by the albumin fraction, is an important source of error, causing up to a 10 per cent reduction in the albumin value. The degree of adsorption has been demonstrated by electrophoresis and radioautography of I^{131} labeled albumin (16). "Trailing" may account for the occasional lower values obtained for albumin by paper as opposed to free electrophoresis. Gamma globulin behaves in a similar way, but no error is introduced with the technique described, owing to its limited mobility. "Trailing" of protein on the paper is largely responsible for the failure of the valleys between the peaks to approach the baseline of the electrophoretic pattern as closely as occurs in free electrophoresis. Thus, the area under the globulin peaks may not entirely represent single fractions, but will contain elements of the faster moving fractions, particularly albumin. When adsorption, or inadequate separation, results in the areas of individual globulin components appearing as "shoulders" rather than discrete peaks, any attempt to resolve the pattern into areas is quite inaccurate.

Noting a lack of agreement between the paper and free electrophoretic analysis of the same serum, previous investigators concluded that a "globulin factor" should be used to equate the results obtained by the two methods. However, there has been no unanimity of opinion as to the magnitude or desirability of such a factor, as evidenced by the following observations: Using bromphenol blue and elution, Cremer and Tiselius (3) observed that globulins appeared to bind less dye than albumin and derived a factor of 1.6 by which the globulin values should be multiplied to achieve comparability with free electrophoretic analysis. Similar factors have been used by subsequent workers employing the dye elution technic (8, 9, 12). Com-

TABLE VI

Comparison, methods of analysis of paper and free electrophoretic components, lipemic sera

Case No.	Biuret total protein *Gm.* %	Total non-dialyzable solids (TNDS)	Components	Paper electrophoresis		Free electrophoresis		
				% planimeter area	Conc. from biuret *Gm.* %	% planimeter area	Conc. from biuret *Gm.* %	Conc. from TNDS *Gm.* %
37	6.23	8.23	Alb	37.6	2.34	30.7	1.91	2.53
Nephrotic			α_1	7.4	0.46	7.8	0.49	0.64
syndrome			α_2	17.9	1.12	22.6	1.41	1.86
Diabetic			β	22.8	1.42	28.8	1.79	2.37
nephropathy			γ	14.3	0.89	10.1	0.63	0.83
40	7.65	10.40	Alb	60.4	4.62	46.0	3.52	4.78
Hypercholes-			α_1	2.7	0.21	5.5	0.42	0.57
terolemic			α_2	7.4	0.57	10.2	0.78	1.06
xanthomatosis			β	14.3	1.09	27.7	2.12	2.88
			γ	15.1	1.16	10.6	0.81	1.10
41	7.40	10.70	Alb	53.4	3.95	40.0	2.96	4.28
Hypercholes-			α_1	2.3	0.17	4.5	0.33	0.48
terolemic			α_2	9.9	0.73	10.5	0.78	1.12
xanthomatosis			β	16.6	1.23	33.3	2.46	3.56
			γ	17.8	1.32	11.7	0.87	1.25

paring albumin and γ globulin fractions alone, Kunkel and Tiselius (5) found that, with a Folin tyrosine-reacting reagent, γ globulin gave a more intense color per unit of nitrogen than albumin; by bromphenol stain and elution, however, the reverse was true, giving a correction factor for globulin of approximately 1.3. Grassmann and Hannig (7) suggested a β globulin factor of 1.25 using their photometric method with amido black. Sommerfelt (42) reported that with amido black followed by elution, it was necessary to use a globulin factor of 1.3, but with the same dye and direct photometry, no factor was required. Griffiths (15), for bromphenol blue and direct photometry, stated that he found no evidence that any globulin factor was required. On the basis of many comparisons between paper and free electrophoresis, using pathological sera and bromphenol blue dye, Köiw, Wallenius, and Grönwall (11) derived separate factors for each individual globulin component ranging from 0.9 to 8.1; it was emphasized, however, that these factors varied greatly from one serum to another.

Our series of comparisons of paper with free electrophoresis indicates that results obtained with the two methods are in good general agreement. The major discrepancies occurred in the β globulin fraction, which often gave lower values with paper electrophoresis. The lipid components of these globulins presumably contribute to the refractive change in free electrophoresis, but do not stain with protein-binding dyes. As would be expected, values for lipemic sera and for sera with a high β globulin peak on free electrophoresis agreed least well.

We have shown that γ globulin also may have a slightly lower dye-binding capacity than albumin; however, values for albumin tend to be reduced, and γ globulin values increased by protein adsorption on the paper. All these considerations lead to the conclusion that paper electrophoresis measures serum protein complexes somewhat differently than does free electrophoresis; hence the use of an empirically derived globulin correction factor is not recommended, as no such factor can exactly equate the results obtained by the two methods.

The electrophoretic alterations in serum of diseased states which affect plasma protein metabolism have been fully reviewed (40, 43–45). The diagnostic value of any type of electrophoretic analysis of serum is limited by the fact that gross alterations of serum proteins in diseased states commonly follow general rather than specific patterns. In only a few situations, as in agammaglobulinemia, can electrophoretic patterns be considered of real diagnostic importance. The advantages of filter paper electrophoresis as compared with free electrophoresis are that it is simple, inexpensive, and requires very small amounts of protein. The technique is especially applicable to very opaque sera which often cannot be analyzed

by free electrophoresis. Our studies indicate that, for normal sera, results obtained with paper and free electrophoresis correspond well. The general protein disturbances in various pathologic conditions such as myeloma, portal and biliary cirrhosis, the nephrotic syndrome, and agammaglobulinemia are very satisfactorily revealed by the paper technique.

Photometric analysis of the paper pattern is much quicker than the more tedious elution method, but it does not seem more accurate than the latter. In most cases, simple inspection of the paper strip will demonstrate clearly the nature of any significant serum protein abnormality present, without any necessity for time-consuming quantitation of the pattern.

SUMMARY

1. Methods for paper electrophoresis using the Grassmann-Hannig and Durrum apparatus are described. Analyses of normal and abnormal sera were made by photometric measure of the serum protein components on the dyed paper strip.

2. Reproducibility of analysis is good, but does not quite meet the accuracy reported by others for free electrophoresis.

3. Results with paper electrophoresis compare well with analyses of duplicate samples by the Tiselius method, except with certain abnormal sera, such as those of high lipid content. Reasons for discrepancies between the two methods are investigated; it is concluded that a correction factor for globulin values should not be used.

4. Analyses of the serum protein changes in a wide variety of pathological states are presented, and the clinical usefulness of the procedure is discussed.

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BONE AS A SODIUM AND POTASSIUM RESERVOIR [1, 2]

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Gamble, Ross, and Tisdall (1) originally pointed out that electrolytes and water were lost from the body during fasting in a manner which could be reasonably predicted from the concentration of salts in the body fluids. This concept is the basis for the study of changes in body composition by examination of electrolyte balance and weight changes. Darrow, Da Silva, and Stevenson (2) elaborated the method of calculation and showed that shifts of sodium between extracellular and intracellular fluid could be inferred in certain conditions from balance measurements. In this laboratory, studies in both man and animals (3, 4) have frequently demonstrated losses or gains of sodium which cannot be reasonably explained on the basis of shifts so calculated. Two possible explanations are: (a) Unmeasured skin losses may lead to the calculation of erroneously large retentions, or (b) Electrolyte may be sequestered in the body in an osmotically inactive form quickly available to the body fluids. Flanagan, Davis, and Overman (5), studying both balances and composition of tissues in dogs with adrenal insufficiency, noted such discrepancies and suggested that bone might serve as a sodium reservoir.

The early work of Gabriel (6) demonstrated the presence of substantial amounts of sodium in the chloride-free residue of bone extracted with alkaline glycol solutions. Harrison, Darrow, and Yannet (7) showed that the ratio of sodium to chloride in the skeleton is greatly in excess of that found in a plasma ultrafiltrate, and defined this as "extra" bone sodium. Subsequent investigators have confirmed these findings (8, 9), and Kaltreider, Meneely, Allen, and Bale (8), Stern, Cole, Bass, and Overman (10), and Edelman, James, and Moore (11), using radioactive isotopes, have shown that approximately 40 per cent of bone sodium is exchangeable with circulating radiosodium within 24 hours.

The chemical relationships of sodium and potassium to the crystal lattice of bone are not surely known. Neuman and associates (12) consider bone to have the general formula of a multiple apatite, $Ca_9(PO_4)_6 \cdot CaX_2$. Their experimental data indicate that, under physiologic conditions, CO_2 appears to be bound to the X positions of such a salt by a single bond—*i.e.*, as bicarbonate. They point out, however, that CO_2 may actually be present as carbonate because of secondary ionization of such a complex: $-Ca-O-C-O_2-H+Na^+$ (or K^+) $\rightleftharpoons -Ca-O-C-O_2-Na(K)+H^+$. Experimental work by one of the authors (13) is in keeping with the concept of secondary ionization.

One of the implications of this concept of the nature of the binding of sodium and potassium to the mineral portion of bone is that the crystal lattice may act as an ionic exchanging area capable of donating sodium ions to the body fluids in exchange for hydrogen ions during periods of acidosis. Since skeletal sodium accounts for approximately a third of the total body sodium (8–10), an investigation of its role in this regard seemed desirable. The experiments to be described here were undertaken to determine the effect of acidosis and of sodium deprivation on the relative quantities of sodium, potassium and calcium in bone.

EXPERIMENTAL PROCEDURE

Acute sodium depletion and acidosis were induced in albino rats (Lansing strain) by intraperitoneal dialysis against a solution containing 50 Gm. of glucose and 90 mM. of ammonium chloride per liter. The amount injected intraperitoneally was 10 ml. per 100 Gm. of body weight. Four hours after injection as much peritoneal fluid as possible was withdrawn. The amount obtained usually exceeded that injected. The animals were then allowed water *ad libitum* but no food in order to pre-

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vent access to exogenous base. After 48 hours the animals were sacrificed.

Chronic sodium deprivation was achieved by raising weanling rats on a synthetic sodium-free diet. The composition of the control ration was that used by Cotlove, Holliday, Schwartz, and Wallace (14); the experimental animals were given the same diet with ammonium ion substituted for sodium in the salt supplement. All of the rats in this series received the complete diet for one week; the experimental group was then given the sodium-free diet and the controls continued on the complete ration. After a two-week experimental period, all animals were sacrificed.

At the time of sacrifice, blood was withdrawn from the aorta for serum analyses. All of the long bones of each animal were removed, freed of periosteum and epiphyses and weighed.

CHEMICAL METHODS

A. *Bone:* (*1*) *Water* was taken to be the difference between fresh wet weight and dry weight after 48 hours at 105°C. The dried bone was ground in a mortar and the powder used for subsequent analyses. (*2*) *Chloride* was determined in duplicate aliquots of dry powder by an adaptation of the method of Sunderman and Williams (15) to semi-micro quantities. (*3*) *Sodium* and *Potassium* were measured using an internal standard flame photometer (16). The very high ratio of calcium to sodium in bone precludes accurate analysis of the sodium even when the internal standard is used. Sodium must be quantitatively separated from calcium for analysis. One hundred to 200 mg. of dry bone powder was ashed in a platinum vessel at 550°C. The ash was dissolved in 11 ml. of 10 per cent nitric acid and 5 ml. aliquots were transferred to 40 ml. centrifuge tubes. Five ml. of 5 per cent oxalic acid and 10 ml. of water were added and the pH brought to 8–9 (bromthymol blue) by running in 3 to 4 ml. of concentrated NH_4OH from a burette. Appreciable quantities of sodium co-precipitate with calcium under these conditions (17), so that repeated precipitations are required for separation. The tubes were allowed to stand overnight and, after centrifugation, the supernatant fluid was transferred to a volumetric flask. The precipitate was then re-dissolved in nitric acid and the process twice repeated. The supernatant fluids from all three precipitations were combined in the same volumetric flask, to which an appropriate quantity of lithium was added before flame photometry was carried out. This method, when applied to solutions containing weighed amounts of sodium, potassium, calcium, magnesium, and phosphorus in the proportions appropriate for bone ash, gave recovery values for sodium and potassium within 1 per cent of the known values. Analysis of multiple aliquots of bone powder indicated reproducibility within plus or minus 4 per cent. It was found necessary to use a reagent blank with each set of determinations, since the C.P. grade nitric acid and ammonium hydroxide used contained significant amounts of sodium and potassium even when redistilled in Pyrex glassware. (*4*) *Calcium* was measured by permanganate titration of the precipitate left from the sodium and potassium analysis, after washing twice with 2 per cent ammonium hydroxide to remove excess oxalate (18). Accuracy and reproducibility were found to be within 1 per cent.

B. *Serum:* (*1*) *Sodium* and *Potassium* were measured with the flame photometer, using methods previously described in this laboratory (16). (*2*) *Chlorides* were done in duplicate by the iodometric method of Van Slyke and Hiller (19). (*3*) *Serum pH* was determined by the method of Hastings and Sendroy (20). (*4*) *Total serum* CO_2 was determined by the method of Van Slyke and Neill (21).

CALCULATIONS

Values for all constituents were expressed as mEq. per kilogram of fresh bone. Chloride was assumed to be extracellular. On this basis, bone water was divided into extracellular and intracellular compartments using Donnan-corrected serum chloride concentrations and total bone chloride values. Total bone sodium was then corrected by subtracting the amount of sodium calculated to be present in the extracellular phase. The remainder was regarded as "extra" bone sodium as originally defined by Harrison, Darrow, and Yannet (*7*). Intracellular water, as approximated above, was arbitrarily assigned a potassium concentration of 150 mEq. per liter. Total bone potassium was corrected for intracellular potassium thus estimated, and the remainder was taken to represent "extra" potassium, analogous to "extra" sodium. The necessity for assuming rather than measuring the potassium concentration of bone marrow cell water is an admitted source of error. The small amount of marrow available in the rat and the technical difficulty of securing sufficient material for analysis without evaporation dictated the compromise.

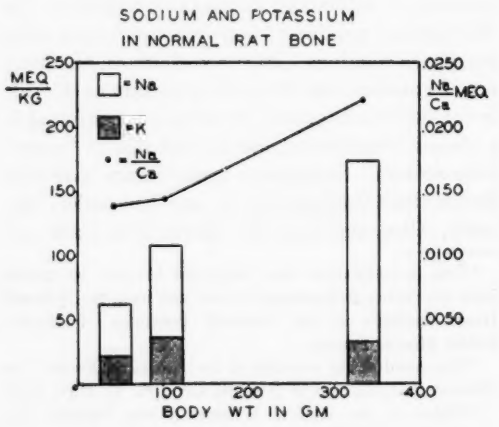

FIGURE 1

TABLE I

Bone and serum electrolyte data, normal, acidotic and low sodium animals

Group*	Av. wt.	Bone								Serum‡			
		Na. *mEq./Kg.*		K. *mEq./Kg.*									
		Total	Corr.	Total	Corr.	Ca	Cl	Na/Ca	K/Ca	pHs 38°	CO_2	Na	Cl
	Gm.					*mEq./Kg.*	*mEq./Kg.*				*mM./L.*	*mEq./L.*	*mEq./L.*
Normal Adults (14 Rats)	333	206	173	43	30	8000	25	.0218	.0028	7.33	26.3	148	109
S.E.		6.2	5.7			313		.0005	.0003	.02 (8)	1.2 (8)		
Acidotic Adults (14 Rats)	265	154	119	22	10	7800	26	.0152	.0013	7.20	20.9	138	97
S.E.		7.1	6.7		1.9	165		.0007	.0002	.02	0.3		
P			.01		.01	.6		.01	.01	.01 (8)	.01 (8)		
Normal Juvenile (8 Rats)	103	157	108	54†	34†	7500	36	.0145	.0055			159	107
S.E.		7.6	8.4			203		.0012					
Acidotic Juvenile (6 Rats)	109	139	87	44†	27†	6200	34	.0139	.0044			157	97
S.E.		5.8	5.8			174							
P			.05			.01							
Weanling (3 Rats)	43		62		21	4400	36	.0140	.0047				
Low Sodium Controls (7 Rats)	141	154	125	99	58	6100	24	.0208				153	106
S.E.		13	13	10	7	165		.0027					
Low Sodium (8 Rats)	109	91	64	43	5	6100	21	.0105				148	105
S.E.		8	8	8	5	222		.0013					
P			.01		.01			.05					

* S.E. and P are calculated according to Fisher's t test.
† Data for 4 rats only.
‡ Numbers in parentheses indicate number of observations where this differs from number of rats in experimental group.

RESULTS

Normal values are shown in the table and in Figure 1. Sodium and potassium contents are corrected as explained above and are expressed as mEq. per kilogram of fresh wet bone; the ratio of each of these cations to calcium in mEq. is also shown. Harrison used the latter method of notation to distinguish between changes in bone salt composition (22). The chart and tables show that bone sodium increases with increasing body size. The absolute increase in sodium (from 62 to 173 mEq. per Kg.) is greater than the increase in the Na/Ca ratio (.0140 to .0220). This is to be expected, since it is known (23) that the ash content of bone is greater in older animals. The change in ratio is consistent with previous demonstrations of change in bone salt composition with age (23, 24). The average corrected sodium content was 173 mEq. per kilogram in adult (333 Gm.) rats; in juvenile (103 Gm.) rats it was 108 mEq. per kilogram. Corresponding potassium values were 30 and 34 mEq. per kilogram.

The results of dialysis against ammonium chloride are shown in Table I and illustrated in Figures 2 and 3. This procedure produced a moderate degree of acidosis as evidenced by significant decreases in serum pH and total carbon dioxide in the adult-sized animals. Determination of the serum acid-base pattern of the smaller animals is technically difficult and no data are available to document acidosis in the juvenile group. Analysis of the dialysate after withdrawal indicated that approximately 15 mEq. of sodium per kilogram of

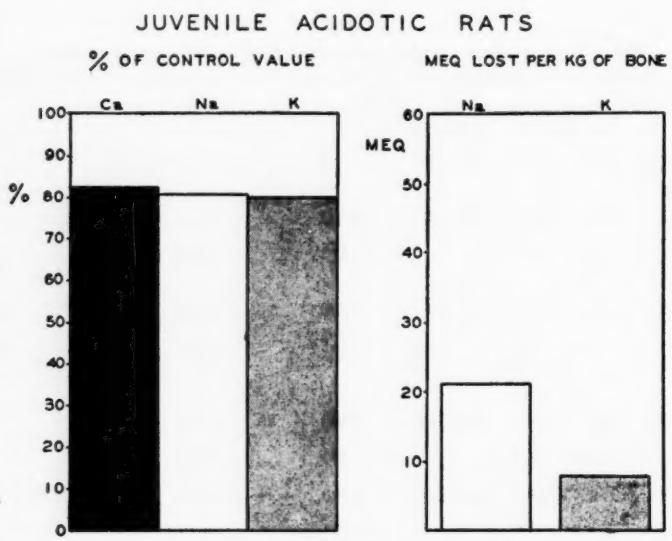

FIGURE 2

body weight were removed from the body by this procedure. The average value for total body sodium in the rat is 60 mEq. per kilogram of fat free weight (25). In the juvenile group the procedure resulted in the loss of 21 mEq. of sodium and 7 mEq. of potassium per kilogram of bone; a proportionate loss of calcium occurred and there was no significant change in the ratio of either cation to calcium. In the adult group there was a loss of 54 mEq. of sodium and 20 mEq. of potassium per kilogram of bone. The change in calcium (200 mEq. or 2.5 per cent) was not significant. The Na/Ca ratio decreased from .022 to .015; K/Ca from .003 to .001. The reason for the difference in reaction of the two groups of animals is not apparent. It has been found that shaft bone sam-

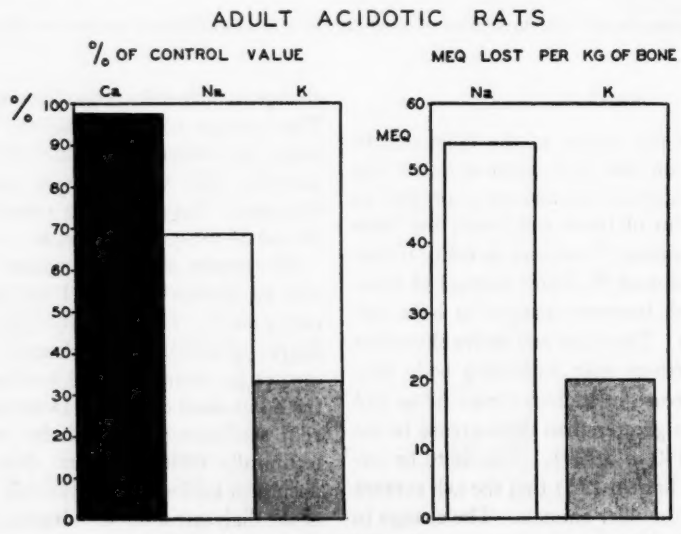

FIGURE 3

ples from young animals have a much more rapid turnover of calcium and phosphorus than corresponding samples from mature animals (12). This suggests that the proportion of bone salt accessible as a source of base may be greater in young animals than in older ones.

The last two groups of animals shown in Table I indicate the effect of chronic dietary sodium ion deprivation on the composition of the bone of rapidly growing animals. The concentration of calcium in the bones of the control and low sodium group are equal indicating little interference with the deposition of this ion. The concentration of sodium is 50 per cent lower in the experimental group than in the controls. An even greater change in the concentration of potassium is evident. The decrease in concentration of potassium in the face of an adequate dietary intake is explained by the fact that diets low in sodium, if they are normal in respect to other ions, contain inadequate quantities of cation to enable full renal regulation of acid-base balance. The effects of a low sodium ion diet cannot, therefore, be differentiated from those of fixed anion excess.

DISCUSSION

Rapid changes in the chemical composition of bone are known to occur during metabolic disturbances. In 1933, Irving and Chute (26) found that bone lost substantial amounts of carbonate in rats and guinea pigs undergoing acute acid loads. After four days of oral HCl administration, their experimental animals showed a decrease in bone carbonate of 7 mEq. per kilogram of body weight, which is equivalent to approximately 70 mEq. per kilogram of bone, since fresh skeletal weight is equal to 10 per cent of body weight in the rat (27). Brooke, Smith, and Smith (28) showed a low salt content in the bones of rats fed on a diet poor in inorganic constituents and noted that the calcium lost on the salt-poor ration ". . . is provided to a greater extent from the carbonate of bone than from phosphate." More recently, Sobel, Rockenmacher, and Kramer (29) found that the calcium to carbonate ratio of bone ash could be changed in rats by altering the serum phosphorus to carbonate ratio.

In 1934, Orent, Kruse, and McCollum (30) studied changes in bone and serum magnesium in weanling rats raised on a magnesium deficient diet. They observed acute lowering of bone magnesium and elevation of serum magnesium when convulsions were induced in the deficient animals. Duckworth and Godden (31) later concluded that the skeleton constitutes the main magnesium reserve of the body and that skeletal magnesium is highly labile. They found that magnesium was more readily mobilized in animals which were deficient in calcium as well as magnesium, and stated that the availability of skeletal magnesium was inversely proportional to the rate of bone growth.

These observations indicate that the composition of bone salt is not fixed, but reflects the state of chemical equilibrium in the body. Severe disturbances in acid-base balance or in the supply of certain electrolytes are reflected in altered bone salt composition. Hence, it is not surprising that significant amounts of sodium and potassium should be mobilized from the skeleton under appropriate circumstances. The earlier investigations mentioned above show that such mobilization may occur without general dissolution of the crystal lattice, *i.e.*, that it may be selective. This is confirmed by the results of the present study, which indicate altogether disproportionate changes in sodium and calcium.

The magnitude of the changes observed may be illustrated by reference to the 70 kilogram man. Skeletal weight in this individual would approximate 12 kilograms, according to the data of Shohl (32). A loss of 54 mEq. per kilogram from bone could, therefore, contribute 648 mEq. of sodium to the body fluids—the equivalent of more than 4 liters of extracellular fluid. This amount of fixed base could combine with nearly 6.5 liters of 0.1 normal anion. A similar calculation for potassium would give 122 mEq., equivalent to 800 ml. of intracellular water.

The lability of bone sodium observed in these experiments is relevant to the interpretation of electrolyte balance data. It is evident that sodium balances cannot always be considered only in terms of extracellular and intracellular water, since substantial amounts of excreted sodium may represent skeletal contributions. The data suggest that bone also participates in potassium exchange, though to a lesser degree. Whether either of these cations can be sequestered in bone to a significant extent during the administration of sodium and po-

tassium salts is not yet known, though the possibility is apparent.

SUMMARY AND CONCLUSIONS

1. Average normal rat bone contains 152 mEq. of sodium and 26 mEq. of potassium per kilogram of fresh bone in excess of the amounts accounted for by extra- and intracellular fluid.

2. Rats depleted of sodium and made acidotic by intraperitoneal dialysis against ammonium chloride showed, within 48 hours, a decrease of bone sodium amounting to 42 mEq. per kilogram of bone and a decrease in potassium of 11 mEq. per kilogram. Changes in calcium were not proportionately large.

3. Rats raised on a synthetic sodium-free diet had bone sodium values 61 mEq. per kilogram below those of controls.

4. No obligatory ratio of either sodium or potassium to calcium was observed.

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A STUDY OF SERUM BETA LIPOPROTEIN AND TOTAL CHOLESTEROL VARIABILITY AND ITS RELATION TO AGE AND SERUM LEVEL IN ADULT HUMAN SUBJECTS [1]

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This study is concerned with an evaluation of the nature and magnitude of the variability of the serum cholesterol and certain classes of serum lipoproteins in adult men. The immediate objectives of the experiments described were three: (*1*) to study the relation of age and mean serum level to the day-to-day variation in serum lipid levels, (*2*) to characterize the magnitude and origin of the variability encountered, and (*3*) to define the number of samples which must be analyzed to yield reliable values.

Despite the abundance of cholesterol in human blood and the many discussions about its significance, descriptions of "normal" levels have been diffuse and often inconsistent. There has been no agreement upon the extent of individual variability with time nor variability in different physiological states. The lack of uniformity in results can be attributed in part to the technical variability or nonspecificity of the difficult analytical methods. It may also reflect a lability of biological control.

The nature of variability in relation to serum and tissue cholesterol as well as to the other lipids measured by chemical methods has been discussed extensively by Bloor (1) and more specifically by Bang (2). The influence of meals and the existence of a diurnal variation of serum cholesterol have been reported by McClure and Huntsinger (3), Bruger and Somach (4), and Boyd (5) among others.

Turner and Steiner (6), using the method of Bloor, Pelkan, and Allen, have reported on variation in total serum cholesterol measured at weekly intervals in ten patients at four periods during 63 weeks of investigation. They noted that the maximum per cent deviation of any single total cholesterol determination from an individual's mean for all values ranged from four per cent (mean 276 mg. per cent) to 15 per cent (mean 280 mg. per cent). The maximum deviation was less than ten per cent in six cases (average of individual means 283 mg. per cent). Steiner and Domanski (7), using the same technique, later reported a mean standard deviation of 8.7 mg. per cent in 15 normals with a mean total cholesterol level of 254 mg. per cent and a mean standard deviation of 24.8 mg. per cent in 15 patients with coronary atherosclerosis with a mean level of 355 mg. per cent. Both groups were studied regularly over a two year period. Man and Gildea (8) studied ten young subjects for periods of three to 48 months and found that the range of total cholesterol for individuals varied from one per cent (mean 237 mg. per cent) to 31 per cent (mean 213 mg. per cent) of the mean. Man and Peters (9) reexamined 16 of the same subjects 10 to 20 years later and presented expanded data describing the variability of cholesterol within individuals on six subjects from the original group which revealed coefficients of variation ranging from 4.9 to 9.9 per cent. Sperry (10) studied 25 young subjects for periods of 28 months and found in 17 cases a variation from the mean of 6.2 per cent or less with a maximum variation of 12.3 per cent. He reported an average of the individual standard deviations of 12.1 mg. per cent. He concluded that an individual has a char-

[1] The laboratory analyses reported in this paper were performed in the Department of Nutrition at the Harvard University School of Public Health and were supported in part by grants in aid from the National Heart Institute, National Institutes of Health, Bethesda; the Nutrition Foundation, New York; the J. M. Kaplan Fund, New York; and the American Meat Institute, Chicago.

acteristic level from which large deviations do not occur.

Schube (11) studied the variations of blood cholesterol of ten male subjects 18 to 35 years of age whose levels were measured in duplicate at weekly intervals over a period of 16 weeks by a modification of the Myers-Wordell procedure applied to whole blood. He found the greatest week to week change in an individual to be 73 mg. per cent (mean 143 mg. per cent) and the maximum deviations from the means of individuals to range from 19 (mean 132 mg. per cent) to 47 (mean 143 mg. per cent) mg. per cent.

The individual variability of serum lipoproteins has not been described in detail. Gofman and his associates (12) refer to this with the statement that two measurements 2 to 90 days apart in approximately 65 individuals revealed "that on a constant diet, a single individual shows a reasonably stable level of concentration of such molecules, whether the concentration is low or high." They justify the adequacy of a single sample drawn at any time of day in this manner.

In another discussion, "standard errors" for the 45 mg. per cent level of Sf 12–20 molecules and for the 243 mg. per cent level of cholesterol were presented by the same workers (13). These values were respectively ± 10 mg. per cent and ± 22 mg. per cent for "biologic variation" and ± 14 mg. per cent and ± 26 mg. per cent for "over-all variation." They derived these values from estimates of "the standard deviation of distribution of levels" among 273 "normal" males and from "coefficients of reliability" based on two determinations in an undisclosed number of individuals whose blood was sampled under conditions and at intervals which were not described. They conclude that a single measurement will place the average individual within ± 14 mg. per cent for Sf 12–20 molecules and within ± 26 mg. per cent for cholesterol of the "true" levels two-thirds of the time. Gofman and his associates (14) subsequently presented values differing only slightly arrived at by a similar technique.

A detailed study of spontaneous and induced variation of serum cholesterol and certain classes of lipoproteins during short time intervals (hours) has been made (15). It was observed that spontaneous variability within an individual was in part dependent upon the level of the particular lipid. Variability at high lipid levels exceeded that of subjects with low levels. In certain individuals, a large biological variation (*i.e.*, exceeding technical variations) was observed between successive bleedings.

For the present studies, a group of adult American males consuming a self-selected diet in habitual amounts and living in a free but medically observed environment to which the subjects were well adjusted was sought. The subjects studied have approached these requirements in most respects.

EXPERIMENTAL PLAN

Three successive studies have been carried out over a period of 13 months. The subjects were, for the most part, groups of adult and of elderly men who were for purposes of this investigation housed in the Hospital for Chronic Illness of the Baltimore City Hospitals. These men were indigent public wards and were not institutionalized primarily because of the presence of disease. In study I, a supplementary group of six adult males, all staff members of the Department of Nutrition at the Harvard University School of Public Health, were studied simultaneously in Boston.

Subjects were excluded who demonstrated prior to the start of the studies diabetes mellitus, definite hepatic insufficiency, the nephrotic syndrome, any known disease of lipid metabolism, hypo- or hyperthyroidism, known malignant disease, an acute infection within two weeks, severe anemia, marked emaciation, marked obesity, active syphilis, active tuberculosis, a surgical procedure within 60 days, hormone or radiation therapy within 60 days, or any anticoagulant therapy with heparin within 60 days. No effort was made to select or reject patients on the basis of atherosclerosis on the grounds that available techniques are inadequate to give more than a very gross estimate of the extent of atheromatous disease. With the exceptions noted in study I, blood was drawn from each subject twice weekly on Monday and Thursday for periods of ten weeks.

In Study I, laboratory measurements were not carried out in duplicate. Both the length of the study period and the frequency of blood samplings varied among the different individuals. In addition, 6 of the 22 subjects were at work in Boston, not institutionalized in Baltimore. Because of these variables, the data in study I will be considered separately. The second and third studies were planned to eliminate these imperfections and the data obtained have been pooled for the present evaluation. Studies II and III were carried out from March to August of 1952.

In the first study, approximately equal numbers of subjects in the age categories 26 to 41, 47 to 58, 63 to 76, and 80 to 92 years were used. In the second and third studies, the age categories of subjects were 47 to 59, 61 to 69,

TABLE I*

Composition and constancy of diet as presented and as actually consumed

	Protein	Fat	Carbohydrate	Calories
	Gm.	*Gm.*	*Gm.*	
Menu analyses	82 ± 3	95 ± 2	262 ± 6	2236 ± 40
Consumption measurements (12–3 d. periods)	60 ± 3	66 ± 5	217 ± 15	1700 ± 113
Average S.E. of consumption (within individuals)	5.7 ± 0.7	6.3 ± 0.8	18.8 ± 1.5	76 ± 17

* The top line shows mean values and standard errors for the components of 28 daily menus. The middle line shows the means and standard errors of the averages for daily consumption during each of twelve three-day periods. The bottom line shows the averages of twelve standard errors of the mean each of which was computed for one of twelve three-day periods.

73 to 79, and 80 to 92 years. An attempt was made to include a range of Sf 12–20 lipoprotein values in each age category. In studies I and II a selection for lipoprotein level was attempted by using a single determination of total cholesterol as performed in the Baltimore City Hospitals clinical laboratory by the Bloor technique (modified) as the sole indicator. In study III, selection for lipoprotein level was based on a single preliminary Sf 12–20 measurement.

Diets. Except for the young adults included in study I, the subjects were confined throughout each study to the hospital wards. They were fed the standard diet provided by the hospital kitchen.

The composition of the diet as presented to the subjects was determined by calculation using food tables of 28 daily menus selected at random during the five-month period of studies II and III. Results of the analysis appear in Table I. Inspection of the standard errors indicates the diet to be remarkably constant from day to day.

During twelve three-day periods in nine subjects, the food actually consumed from the diet presented was recorded and its composition estimated using food tables. For each of the twelve three-day periods a mean daily consumption and the standard error of this mean were computed. The average of the mean intakes and the average of the standard errors for all twelve periods appear in Table I. These values indicate that the average subject did not consume all of the food presented him but that actual food consumption within individual subjects varied little from day to day.

Laboratory. The blood samples were drawn prior to breakfast and at least 14 hours after the last meal. The clotted blood was allowed to retract at room temperature for two and one-half hours. The clots were ringed with clean glass stirring rods and the tubes centrifuged for 15 minutes at 2000 rpm.

After the serum was separated, an aliquot of six ml. was placed in a randomly numbered screw top vial, packed in an ice-filled Dewar flask, and immediately shipped to Boston by air for analysis. During studies II and III, the serum obtained was divided into two six ml. aliquots, each aliquot was given a randomly selected code number, and the individual aliquots sent to the Boston Laboratory ostensibly as separate sera. The analytical results corresponding to each code number were returned to Baltimore for decoding. This technique permitted a truly unbiased estimate of technical error.

The total cholesterol content of serum was measured by the method of Abell, Levy, Brodie, and Kendall (16). Recrystallized cholesterol, with a melting point of 147 to 149° C., was used as a standard reference material measured with the samples processed each day. The serum lipoproteins of the Sf 12–20, Sf 21–35 and Sf 35–100 classes were measured by the technique of Gofman and his associates (17). No feasible reference substance was at hand for standardization of this procedure. During the course of this work, duplicate aliquots of ten serum samples were exchanged between the four laboratories of the Cooperative Study of Lipoproteins and Atherosclerosis at approximately monthly intervals. These data revealed no major deviations of either the cholesterol or lipoprotein methods used in the present study. Instrument calibration with a standard reference cell further minimized the likelihood of the existence of a technical drift in the lipoprotein measurement during the study.

ANALYSIS OF DATA

After decoding, the data were tabulated and the mean level and standard deviation of the distribution calculated for each lipoprotein fraction and for the total cholesterol for each subject. The means ($\bar{x}$) and standard deviations (S_T) were computed from single analyses for 22 subjects in study I and 13 subjects in study III and from the average values of duplicate pairs in 33 subjects on whom these were available in studies II and III.[2]

[2] Treatment of missing data: In those few instances where one value of a duplicate pair was missing (laboratory accident, breakage in transit, etc.) the missing member was considered equal to the remaining member of the pair in computation of S_T. Estimates of S_s were computed in these instances using $n-1$ rather than n pairs, since no value for the difference between duplicates could be assumed in the absence of one member of a pair.

Exclusion of Data: In the 35 subjects on whom only single measurements were made, means and standard deviations were computed using all available data. No datum was discarded even though it could reasonably be expected to be an outlier.

No lipoprotein data were excluded because of missing cholesterol data. Five lipoprotein values were excluded because of recorded technical errors in preparative centrifugation and photographic records of errors in ultracentrifugation. Since it was possible to exclude outliers among lipoprotein values on the basis of these records, it was not considered advisable to process further for outliers. In the case of cholesterol measurements no such permanent records were available, so that testing for outliers among values obtained in duplicate was done statistically using the extreme deviate test (18).

In this latter group a difference between duplicate cholesterol determinations which was demonstrated to be an outlier at the one per cent level of significance resulted in both members of that duplicate pair being discarded, even though one of the values may have agreed well with other data on the individual under consideration. There were 19 pairs of cholesterol observations so discarded out of a total of more than 600. No other cholesterol data were discarded even in the absence of lipoprotein data for a given sample.

The variance among duplicates (S_E^2) was computed from duplicate measurements for each of these 33 subjects and was used for adjusting the individual's total variance. In this way an estimate of residual or what we shall call "biologic variation" (S_B) was obtained. The computation for this adjustment was performed according to the formula:

$$nS_B^2 = nS_T^2 - \tfrac{1}{2}\, nS_E^2$$

For the 35 subjects in studies I and III on whom measurements in duplicate were not available, a S_E^2 used in correcting these individuals' total variance for technical error was arrived at by using the pooled variances among duplicates on those subjects having mean levels of the lipid fraction measured within ten per cent of the level to be corrected. To facilitate this correction, charts

TABLE II

Study I: Data for individuals and age groups

Subject	Age (yrs.)	Weight (Kg.)	Height (cm.)	Beta lipoproteins										Total cholesterol			
				n	S_f12–20 (mg. per cent)			S_f21–35 (mg. per cent)			S_f35–100 (mg. per cent)			n	(mg. per cent)		
					$\bar{x}$	S_T	S_B	$\bar{x}$	S_T	S_B	$\bar{x}$	S_T	S_B		$\bar{x}$	S_T	S_B
J. Dg.	26	72.7	163	8	28.00	6.87	5.59	17.75	6.34	5.90	30.12	12.04	10.77	8	191.1	9.98	—
L. F.	28	76.8	175	7	45.43	9.50	8.52	32.57	7.76	6.93	70.14	19.38	17.06	7	251.0	12.86	—
T. V.	31	70.4	173	10	19.90	6.23	4.97	12.20	2.04	—	22.20	11.08	9.80	10	219.0	15.94	10.30
G. M.	34	64.5	165	9	19.44	3.21	—	10.33	2.83	1.60	19.00	9.27	7.91	9	220.4	8.26	—
D. H.	37	56.8	172	8	52.50	6.16	3.61	23.89	5.58	4.92	30.11	12.39	11.16	9	263.6	15.73	—
F. S.	41	68.2	178	10	35.50	6.20	4.70	18.10	5.34	4.81	39.30	16.54	15.64	10	263.0	25.04	18.91
$\bar{X}$*	32.8	68.23	171.0		33.46	6.36	4.57	19.14	4.98	4.03	35.14	13.45	12.06		234.69	14.64	4.87
$S_{\bar{X}}$†	2.30	2.84	2.38		5.54	2.59	1.14	3.32	2.79	1.09	7.57	1.54	1.44		11.90	2.43	3.27
T. Bt.	47	68.7	173	7	45.71	11.57	10.78	23.29	6.58	6.03	32.57	18.70	17.91	9	160.8	8.94	—
G. S.	48	59.8	170	16	27.38	6.11	4.71	19.12	4.46	3.80	25.69	11.51	10.28	15	174.5	11.91	1.46
E. He.	52	54.5	170	17	31.12	7.66	6.50	19.41	4.35	3.67	38.18	14.38	13.33	17	263.4	25.85	19.97
F. P.	58	65.8	170	16	19.81	4.20	1.89	10.94	2.79	1.54	8.19	5.17	1.81	16	133.5	19.61	15.65
T. Q.	58	52.7	164	14	16.21	3.19	—	8.57	4.09	3.51	12.93	8.18	7.01	14	289.1	19.37	10.30
$\bar{X}$	52.6	60.30	169.4		28.05	6.55	4.78	16.27	4.45	3.71	23.51	11.59	10.07		204.26	17.14	9.48
$S_{\bar{X}}$	2.36	3.10	1.47		5.15	4.66	1.87	2.78	1.93	0.71	5.70	2.36	2.74		30.39	3.01	3.89
W. Re.	63	62.5	165	16	26.81	6.35	4.94	15.19	5.60	5.03	24.63	12.34	11.21	16	219.8	15.25	9.18
B. S.	64	54.2	159	17	18.12	4.96	3.31	8.65	2.85	1.93	13.71	5.84	3.27	17	186.1	14.18	7.27
H. C.	65	59.7	168	16	24.25	5.32	3.53	13.94	3.13	2.09	20.00	6.89	4.55	16	205.9	16.68	11.41
P. K.	73	55.5	162	17	36.06	10.30	9.47	18.41	5.75	5.25	27.88	7.27	5.11	16	164.8	12.56	4.24
J. Me.	73	57.1	159	8	20.75	4.83	2.73	19.62	3.89	3.11	49.88	14.31	13.26	9	197.1	13.76	6.42
F. H.	76	51.1	168	17	28.65	5.52	3.82	13.50	4.23	3.53	20.88	10.25	8.85	17	171.9	30.70	28.34
$\bar{X}$	69.0	56.68	163.5		25.77	6.21	4.63	14.86	4.24	3.49	26.16	9.40	7.71		190.92	17.19	11.14
$S_{\bar{X}}$	2.30	1.65	1.69		2.59	2.68	1.01	1.60	1.57	0.58	5.13	1.41	1.64		8.50	2.76	3.58
F. B.	80	59.1	165	17	37.47	9.15	8.20	18.76	4.59	3.95	68.88	16.31	14.41	16	232.9	14.82	—
C. H.	85	53.2	163	16	36.81	8.50	7.47	26.31	7.25	6.76	65.06	29.15	28.13	17	246.8	22.94	16.04
B. M.	86	52.7	155	6	83.83	14.41	12.48	28.00	5.29	4.59	55.67	13.75	11.93	8	237.9	12.14	—
J. Mi.	91	51.8	163	16	26.75	5.94	4.40	10.31	3.54	2.66	15.69	8.93	7.51	17	183.8	12.14	—
J. W.	92	56.8	156	16	37.50	7.52	6.33	16.44	4.93	4.34	19.19	10.94	9.81	16	196.8	16.11	10.56
$\bar{X}$	86.8	54.72	160.4		44.47	9.10	7.78	19.96	5.12	4.46	44.90	15.82	14.36		219.61	15.63	5.32
$S_{\bar{X}}$	2.18	1.39	2.04		10.05	4.53	1.34	3.26	1.92	0.66	11.43	3.56	3.63		12.36	1.98	3.37

* Average of all values within an age group.

† Standard error of the mean of all values within an age group.

TABLE III

Studies II and III: Data for individuals and age groups

Subject	Age (yrs.)	Weight (Kg.)	Height (cm.)	Beta lipoproteins										Total cholesterol			
				n	S_f12–20 (mg. per cent)			S_f21–35 (mg. per cent)			S_f35–100 (mg. per cent)			n	(mg. per cent)		
					$\bar{x}$	S_T	S_B	$\bar{x}$	S_T	S_B	$\bar{x}$	S_T	S_B		$\bar{x}$	S_T	S_B
A. Hr.*	47	71.2	176	20	21.02	5.86	4.99	12.52	3.66	3.40	37.85	12.83	12.11	18	202.7	11.28	9.45
V. E.	48	68.7	154	17	36.24	5.39	3.40	16.35	4.26	3.51	29.41	10.31	8.82	19	239.1	13.67	—
J. Ca.	50	62.2	171	19	25.50	3.62	—	13.00	3.54	2.61	24.84	8.42	6.52	19	210.3	17.46	12.19
W. C.*	51	69.0	169	19	26.26	5.51	4.26	15.90	3.36	2.45	28.74	7.56	5.41	19	205.8	16.94	14.14
B. C.	51	76.1	162	20	12.10	4.13	1.50	2.60	1.73	—	3.00	3.11	—	20	167.0	31.65	29.24
L. T.	52	62.4	165	20	39.90	8.11	6.96	18.55	3.83	2.99	28.40	6.89	4.40	20	259.8	17.67	5.36
E. Ha.*	53	58.9	171	20	58.22	8.49	6.82	37.20	6.39	5.83	66.05	16.86	15.86	19	242.5	15.41	11.99
J. Br.*	55	70.3	172	19	30.92	6.65	6.29	17.53	6.52	6.30	23.68	10.32	9.63	17	160.0	10.59	7.27
W. F.	55	46.9	175	20	26.40	5.84	4.17	11.65	3.31	2.29	12.45	4.82	—	20	177.2	31.00	28.37
J. Cz.*	57	66.7	171	20	88.38	14.82	13.87	38.98	7.98	7.48	55.22	13.08	11.80	20	271.2	33.41	27.76
L. P.*	59	64.8	165	20	66.62	6.52	4.25	42.00	7.97	7.44	51.42	15.04	14.06	20	159.1	10.93	7.83
$\bar{X}$†	52.5	65.2	168.4		39.23	6.81	5.14	20.57	4.78	4.03	32.82	9.93	8.06		208.61	19.09	13.96
$S_{\bar{X}}$‡	1.1	2.2	1.9		6.87	0.91	1.08	3.88	0.63	0.72	5.62	1.29	1.60		12.13	2.62	3.03
C. G.*	61	62.0	173	20	20.22	3.68	2.67	10.98	2.86	2.59	18.98	8.56	7.82	19	177.2	32.15	30.41
W. A.*	63	60.3	164	20	26.92	3.88	3.10	17.40	3.08	2.21	43.70	10.03	9.07	20	211.0	16.74	13.69
J. Mk.*	63	91.5	171	20	49.90	8.56	7.41	22.10	4.54	4.14	63.75	14.99	14.08	18	195.2	20.16	19.45
R. B.*	64	62.6	168	20	54.90	6.03	5.11	25.02	4.27	3.60	32.72	7.79	6.87	19	144.2	14.02	13.03
J. R.*	64	69.0	166	20	66.42	10.49	9.75	15.68	4.39	3.97	19.28	5.75	4.64	20	204.8	13.44	5.41
J. P.*	65	57.7	175	20	50.25	4.82	4.06	18.68	4.75	4.47	23.50	5.63	4.95	19	202.0	11.89	9.07
H. W.	66	50.0	166	20	33.25	6.43	4.90	11.75	3.43	2.46	24.50	8.11	6.14	20	205.0	22.29	18.46
L. Bk.*	67	47.0	170	19	33.68	9.09	8.89	19.16	4.54	4.16	41.29	11.16	10.49	19	231.7	25.30	22.88
P. D.*	67	61.8	164	20	41.15	8.95	8.40	22.05	7.35	7.25	26.30	9.12	8.81	19	202.6	15.54	14.76
W. L.*	68	56.1	167	20	54.48	8.36	7.59	11.28	3.27	2.92	14.70	4.36	1.58	20	228.0	12.50	8.03
W. T.*	68	73.1	171	19	52.55	6.70	5.25	25.55	7.85	7.55	34.60	15.38	14.85	17	259.1	31.09	28.78
T. R.	69	53.4	173	20	30.55	6.69	5.24	17.75	4.92	4.30	26.85	7.94	5.90	20	148.8	29.83	27.25
$\bar{X}$	65.4	62.1	168.9		42.86	6.97	6.03	18.12	4.60	4.14	30.85	9.07	7.93		200.80	20.41	17.60
$S_{\bar{X}}$	0.7	3.4	1.1		4.02	0.62	0.67	1.46	0.45	0.49	3.93	0.99	1.11		9.43	2.18	2.42
H. K.*	72	49.3	168	20	50.15	6.51	5.74	17.98	3.82	3.51	23.80	8.08	7.48	20	204.8	16.26	14.20
J. Ba.*	73	65.7	166	20	18.45	3.06	0.23	12.68	2.39	1.93	19.70	5.49	4.34	20	170.2	22.67	16.47
A. Hn.*	73	74.4	164	20	33.08	9.05	8.40	25.70	5.60	5.23	65.22	17.02	16.00	19	197.6	25.50	21.21
J. By.*	73	61.2	173	20	23.52	4.90	3.80	14.48	4.29	4.02	18.35	6.37	5.70	20	167.7	18.16	15.75
A. K.	74	65.2	165	20	23.30	4.45	1.75	16.45	2.19	—	40.05	4.77	—	20	204.7	12.40	—
P. B.*	75	73.1	158	20	48.30	7.26	6.82	20.82	4.14	3.73	52.82	15.68	15.26	19	182.6	16.31	15.46
J. S.*	75	49.9	161	20	57.62	10.84	10.18	21.32	3.25	2.37	36.08	5.46	3.36	20	323.3	22.31	8.56
A. A.*	77	58.6	168	20	48.75	6.46	5.82	21.43	3.21	2.91	34.07	6.65	5.88	14	275.1	18.66	13.03
J. Fr.*	77	45.5	161	20	35.75	6.16	4.95	14.80	3.61	2.93	17.00	7.22	6.09	19	153.5	18.15	15.49
J. Co.	78	61.9	170	19	27.16	4.98	2.83	13.79	2.82	1.49	20.95	5.54	1.57	20	205.8	12.49	—
W. D.*	78	81.5	166	20	75.82	14.61	13.15	44.55	5.58	3.97	90.92	18.26	15.99	20	153.7	15.90	13.53
J. Dk.*	79	65.7	163	20	45.90	5.54	5.03	39.15	8.98	8.68	110.55	31.36	30.86	19	182.7	18.58	17.40
$\bar{X}$	75.3	62.7	165.2		40.65	6.98	5.72	21.93	4.16	3.40	44.13	10.99	9.38		201.81	18.12	12.59
$S_{\bar{X}}$	0.7	2.2	1.2		4.86	0.92	1.04	2.92	0.54	0.62	8.81	2.33	2.51		14.49	1.13	1.90
H. B.*	80	73.2	171	20	70.08	7.90	6.81	27.05	5.05	4.68	49.00	9.13	8.36	20	234.7	7.44	—
R. P.*	80	43.1	161	20	15.58	3.05	1.93	9.72	3.01	2.60	15.80	6.95	6.05	18	139.2	11.62	9.70
L. S.*	81	75.9	169	20	50.25	5.25	4.07	32.25	5.33	4.54	119.05	22.12	20.40	20	192.9	14.02	11.19
W. Ra.*	81	83.3	170	20	20.18	4.05	3.09	10.72	2.01	1.31	24.02	5.87	4.81	20	142.9	11.92	10.68
J. T.	81	62.7	172	20	39.20	7.72	6.50	15.10	3.60	2.69	23.20	8.22	6.28	20	218.2	18.46	13.60
J. G.*	82	66.4	168	20	27.58	4.38	3.17	16.90	3.71	3.55	27.02	12.05	11.18	20	174.8	21.90	19.31
M. C.	85	58.7	159	20	35.40	6.34	4.78	24.45	3.19	1.69	40.90	6.77	3.90	20	140.7	15.77	10.08
J. Fa.	85	55.8	166	19	25.53	4.71	2.32	14.26	4.77	4.13	35.47	14.29	13.17	20	189.0	18.30	13.38
J. Mc.	85	53.3	162	19	48.95	8.84	7.72	16.58	3.39	2.39	25.05	8.00	5.98	20	210.0	25.81	22.58
G. A.*	87	73.6	159	20	38.68	5.09	3.96	17.82	2.16	0.89	27.15	5.56	3.50	20	207.9	16.89	14.06
L. Ba.*	92	53.0	159	20	45.98	8.03	7.55	32.38	6.11	5.72	48.85	14.20	13.65	19	155.1	11.87	10.61
$\bar{X}$	83.5	63.5	165.1		37.95	5.94	4.72	19.75	3.85	3.11	39.59	10.29	8.84		182.31	15.82	12.29
$S_{\bar{X}}$	1.1	2.7	1.0		4.72	0.58	0.63	2.42	0.40	0.46	8.58	1.51	1.58		10.25	1.58	1.73

* Values derived from duplicate determinations.
† Average of all values within an age group.
‡ Standard error of the mean of all values within an age group.

relating the variance among duplicates to mean levels for each of the four lipid components were constructed. The correction was then performed in each of these 35 subjects according to the formula:

$$nS_B^2 = nS_T^2 - nS_E^2$$

Regression equations were computed relating the standard error among duplicate analyses (S_E) to mean level, over-all variability (S_T) to mean level, and biological variation (S_B) to mean level. Correlation coefficients were computed relating mean cholesterol levels to mean levels of Sf 12–20, Sf 21–35, Sf 35–100 molecules and to the sums of these mean levels (Sf 12–100).

The data obtained during study I appear in Table II. The mean values for individuals as well

as for groups of individuals for the age categories described above are shown along with measures of the variability including the biological variability (S_B) computed according to the procedures described. Similar data for studies II and III are shown in Table III.

Since subjects were selected for study to provide among other things a wide range of lipoprotein levels in each age group, sampling was not strictly random. Thus in order properly to compare the S_T (and S_B) among age groups some sort of adjustment for lack of randomness in sampling levels was necessary. This adjustment was made for studies II and III combined by the statistical technique known as the analysis of covariance. This involves verifying that the regressions of S_T (and S_B) on level have the same slope in each age group; if such is the case, it is then possible to make a regression adjustment for each value of S_T (and S_B) before testing age group averages for homogeneity. The results of the preliminary analysis implied that all within-age regressions for studies II and III had substantially the same slope.

The analysis of covariance was not performed for study I because of the various defects of that study previously described. Consequently the regression equations and statistical tests for study I should be viewed with some reservations. It is, however, interesting to note that at least in terms of absolute differences between pairs of slopes and intercepts, the two sets of regression equations are much the same.

Analysis of adjusted age group differences showed a statistically significant agewise difference in variability ($P = .05$) only in the case of the Sf 21–35 fraction. However, in this last case, since computation showed a difference of approximately one mg. per cent between the upper two and lower two age groups, there seemed, as a practical matter, little point in supplying separate regressions for each age class.

TABLE IV

Regression analysis of S_T on $\bar{x}$ for various lipoproteins and total cholesterol

Study	n	Lipid	Regression*
I	22	S_f12-20	$S_T = 2.19 + .147\bar{x}$
		S_f21-35	$S_T = 1.52 + .181\bar{x}$
		$S_f35-100$	$S_T = 5.21 + .225\bar{x}$
		Total Cholesterol	Not Significant
II & III	46	S_f12-20	$S_T = 2.00 + .116\bar{x}$
		S_f21-35	$S_T = 1.48 + .142\bar{x}$
		$S_f35-100$	$S_T = 2.86 + .196\bar{x}$
		Total Cholesterol	Not Significant

* Slopes significant, $p < .001$.

TABLE V

Regression analysis of S_B on $\bar{x}$ for various lipoproteins and total cholesterol

Study	n	Lipid	Regression*
I	22	S_f12-20	$S_B = 0.24 + .157\bar{x}$
		S_f21-35	$S_B = 0.36 + .202\bar{x}$
		$S_f35-100$	$S_B = 3.57 + .228\bar{x}$
		Total Cholesterol†	
II & III	46	S_f12-20	$S_B = 0.26 + .128x$
		S_f21-35	$S_B = 0.75 + .146\bar{x}$
		$S_f35-100$	$S_B = 1.02 + .204\bar{x}$
		Total Cholesterol	Not Significant

* Slopes significant, $p < .001$.
† Since in 8 of the 22 cases the total variance for cholesterol was less than technical variance, computation of regression was not attempted.

As a consequence of the analysis described above, it was considered justifiable to compute over-all regression lines of S_T (and S_B) on level for the various measures considered. For all of the lipoproteins, the regression slopes were significant at the .001 level. A significant regression of S_T (and S_B) on mean level of total cholesterol could not be demonstrated. (Tables IV and V).

A highly significant regression was demonstrated between the standard errors among duplicates and the mean levels of both the lipoprotein fractions and the total cholesterol in the 33 subjects from studies II and III on whom duplicate analyses were available (Table VI). Regression equations indicate that the standard error among duplicates increases about 0.06 mg. per cent for each mg. per cent increment in mean level for all the lipoprotein fractions and about 0.08 mg. per cent for each mg. per cent increment in total cholesterol.

The subjects were selected both for age and

TABLE VI

Regression analysis of S_E on $\bar{x}$ of lipoproteins and total cholesterol ($n = 33$)

Lipid	Regression*
S_f12-20	$S_E = 2.35 + .052\bar{x}$
S_f21-35	$S_E = 1.23 + .065\bar{x}$
$S_f35-100$	$S_E = 3.45 + .061\bar{x}$
Cholesterol	$S_E = -2.81 + .079\bar{x}$

* Slopes significant, $p < .001$.

serum lipid levels according to the several procedures described above. It was observed that the single determination of serum cholesterol on each subject performed in the clinical laboratory of the Baltimore City Hospitals was significantly correlated with the mean total cholesterol level of that individual for the entire study but this single cholesterol measurement did not successfully predict the mean level of the lipoprotein classes. The initial Sf 12–20 measurement effectively predicted the mean level of Sf 12–20 and Sf 21–35 but not Sf 35–100.

An examination of the relations between mean levels of total cholesterol and mean levels of various lipoproteins revealed no correlations significantly greater than zero in study I and a correlation significantly greater than zero only in the case of Sf 12–20 molecules in studies II and III.

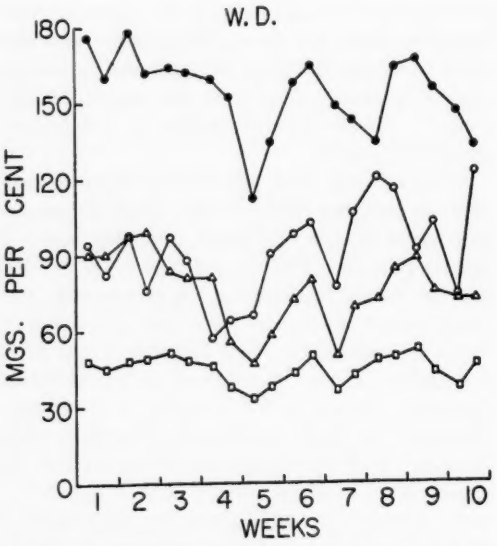

FIG. 1. VARIATIONS IN CHOLESTEROL AND CERTAIN LIPOPROTEIN LEVELS EXAMINED TWICE WEEKLY FOR A TEN WEEK PERIOD

Patient W. D., age 78. Triangles, Sf 12–20; squares, Sf 21–35; open circles, Sf 35–100; closed circles, total cholesterol.

DISCUSSION

These investigations provide information about several kinds of variability for three classes of beta lipoproteins and of total cholesterol among individuals who were living in a controlled environment with no appreciable variation in activity and diet from day-to-day. Their failure to demonstrate significant differences in variability among various age groups implies that the homeostatic mechanisms for these serum lipids may not undergo alteration with age. While variability could not be related to age directly it is apparent that the characteristic increase of lipid levels with age to a maximum in the sixth decade (13, 19) would indirectly lead to some change in variability with age. Since subjects were selected for these studies to provide a similar range of Sf 12–20 levels in each age category, the usual age trends in level were not observed.

These studies indicate that technical error (S_E) and biologic variability (S_B) (and consequently over-all variability (S_T)) increase with the mean level among individuals. Inspection of available clinical information obtained during the ten-week period of these studies revealed no explanation for the origin of this biologic variability. Changes in body weight (trivial in this situation), changes in environmental temperature, intercurrent upper respiratory infections, and differences among individuals in physical activity were among the influences considered.

In addition to these net variations of serum lipid quantities within an individual over a 10-week period, another aspect of this variation deserves comment. This is the tendency of some individuals to show trends of change, either unidirectional or at times suggesting a periodic phenomenon. Such variations are obscured by the condensation of individuals' data into means and measures of range of single measurements. An example of this variation is shown in Figure 1. Attempts have been made to reduce these phenomena to some order by the application of one or another of the available statistical techniques for dealing with nonrandom changes. When tests for serial correlation (20) between successive bleedings were applied to the Sf 12–20 data of 46 subjects for whom complete sets of data for the 10-week study periods were available, there were five subjects with serial correlation coefficients significantly different from zero at the five per cent or less level of probability. This frequency (or a greater frequency) of significant coefficients is of

small enough probability ($p = 0.08$) to suggest that there may be real trends in certain individuals. These are not now explicable but their existence must be recognized as indeed it must be in any body of serial biologic observations. The hazard which their presence lends to the interpretation of either diagnostic or therapeutic studies and for that matter any statistical analysis must be appreciated.

From a practical standpoint the data acquired in this investigation provide quantitative information on the magnitude, at any given serum level, of the overall variation, the technical error and the biological variability of the lipids studied. It is implicit in the regressions of S_T, S_E, and S_B on mean lipoprotein levels that high levels are subject to greater fluctuation than low. From this it may be inferred that caution should be exercised in interpretations of diagnostic, prognostic or therapeutic importance based on values obtained from single or a limited number of blood samples from a given individual, especially when these values are elevated. With respect to total cholesterol, it is of interest that S_E increases with level but that S_T and S_B do not.

The data also permit estimation of the number of samples (n) required to yield an average level of any desired probability within any specified distance (d) of the true mean. This is accomplished by determining n from the relation $n = \frac{S_T^2 t^2}{d^2}$, where S_T is the standard deviation obtained from the regression of S_T on $\bar{x}$ and t is the standardized normal deviate exceeded in absolute value any desired percentage of the time. Sample sizes (n) from a given individual required for a 67 per cent probability ($t_{.33}$) that the average level will be within a distance (d) of the true mean are tabulated for three classes of lipoproteins and for total cholesterol in Table VII. Inspection of the 50 mg. per cent level for Sf 12–20 molecules indicates a single sample adequate to insure a value within ten mg. per cent of the true mean 67 per cent of the time. A sample size of three would insure a value within five mg. per cent of the mean 67 per cent of the time. Inspection of the table for cholesterol, where S_T is essentially the same for all levels, indicates a single sample adequate to insure a value within 15 mg. per cent of the true mean 67 per cent of the time. A sample size of two would insure a value within ten mg. per cent of the true mean 67 per cent of the time. These examples assume that the values for S_E are comparable to those observed in this laboratory and that our estimate of S_B is a reasonable approximation of the true standard deviation of distribution of the measure involved.

Levels of 50 mg. per cent for Sf 12–20 molecules and 250 mg. per cent for total cholesterol may be thought of as levels roughly separating the

TABLE VII

Number of samples (n) required for 67 per cent probability that the observed average level will fall within the distance (d) of the true level

Material	$\bar{x}$ mg. %	S_T mg. %	d mg. %				Material	$\bar{x}$ mg. %	S_T mg. %	d mg. %				
			2.5	5	10	15				2.5	5	10	15	20
S_f12–20	10	3.16	2	1	1	1	S_f35–100	10	4.82	4	1	1	1	1
	20	4.32	3	1	1	1		20	6.78	8	2	1	1	1
	30	5.48	5	2	1	1		30	8.74	13	4	1	1	1
	40	6.64	8	2	1	1		40	10.70	19	5	2	1	1
	50	7.80	10	3	1	1		50	12.66	26	7	2	1	1
	60	8.96	13	4	1	1		60	14.62	35	9	3	1	1
	70	10.12	17	5	2	1		80	18.54	55	14	4	2	1
	80	11.28	21	6	2	1		100	22.46	81	21	6	3	2
S_f21–35	5	2.19	1	1	1		Total Cholesterol	Any	13	28	7	2	1	1
	10	2.90	2	1	1									
	15	3.61	3	1	1									
	20	4.32	3	1	1									
	25	5.03	5	2	1									
	30	5.74	6	2	1									
	40	7.16	9	3	1									
	50	8.58	12	3	1									

normal from the abnormal (14, 19). This separation is most frequently attempted in the fourth, fifth, and sixth decades of age when the level and variability of the individual tested are near their maximum.

To obtain an indication of the accuracy with which a single measurement (Sf 12–20, cholesterol, etc.) can be placed on the continuum of measurements of individuals, an index formed from the ratio of S_T to S_d (the standard deviation of distribution of measurements of individuals) may be used. For an estimate of S_d, the standard deviation of distribution of measures for all 68 subjects has been used. Because of the selection of these individuals to maximize spread of the measures, S_d is probably an overestimate of σ_d (universe value), thus underestimating the ratio σ_T/σ_d. The value of the index of precision for Sf 12–20 molecules at the 50 mg. per cent level is .47 (7.80/16.70); for cholesterol at the 250 mg. per cent level, .32 (13/40.25). This implies that a single sampling of Sf 12–20 will locate an individual within .47 of a S_d 67 per cent of the time; of cholesterol, within .32 of a S_d 67 per cent of the time. Thus cholesterol appears a slightly more accurate measure of "true" cholesterol level than Sf 12–20 is of "true" Sf 12–20 level. From this the inference may be drawn that interpretations based on single samples of Sf 12–20 should be slightly more cautious than these based on a single sample of cholesterol.

There is no indication from these studies that a positive correlation exists between total cholesterol level and the levels of any or all of the lipoprotein molecules measured. This may be due to bias in the sampling (selection for level) or may reflect an inadequate sample size for showing significant correlation (especially if the correlation is low).

SUMMARY

The nature of the variability of the serum cholesterol and certain classes of beta lipoproteins has been studied in 68 adult males. The subjects were for the most part institutionalized for indigence not sickness and were living under known conditions of diet and activity. They were selected to represent a variety of ages and serum lipid levels.

These studies indicate the magnitude of overall variability, of technical error and of biologic variability for three classes of lipoproteins and for total cholesterol. They fail to demonstrate any change in variability related directly to the age of the subject but they do indicate a rise in technical error with mean level for all fractions, including cholesterol, and a linear relationship of the total and biologic variation to the mean level for lipoproteins. They provide data from which an estimate of sample size necessary to provide an average level of any desired probability within any specified distance of the true mean may be obtained. Certain limitations of single measurements of these serum quantities are pointed out, in particular, single measurements of Sf 12–20 lipoproteins at levels above 50 mg. per cent must be interpreted with caution.

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THE EFFECT OF L-NOREPINEPHRINE ON CARDIAC OUTPUT IN THE ANESTHETIZED DOG DURING GRADED HEMORRHAGE [1]

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Clinical reports have recently suggested that l-norepinephrine is a valuable agent in the treatment of shock (1–3). It has been found relatively more effective in those cases in which there is no major decrease in blood volume, such as in shock due to drug sensitivity, myocardial infarction, overwhelming infection and post-operative hypotension unresponsive to transfusion. Also, some cases of surgical or oligemic shock are reported in which it has been used with success (4–6). The rationale for the use of l-norepinephrine in shock rests on the observation that its administration is followed by restoration of blood pressure and general clinical improvement. However, since there is a body of surgical opinion which holds that pressor drugs are contraindicated in the treatment of hemorrhagic hypotension, the present series of experiments was designed to define the circulatory responses of the dog to l-norepinephrine during graded hemorrhage. Cardiac output, arterial pressure and total peripheral resistance were the parameters measured.

The effects of l-norepinephrine on the normal human circulation are well known (7). The drug elicts a bradycardia, an increase in systolic and diastolic arterial pressures, an increase in total peripheral resistance, a slight decrease or no change in cardiac output, an increase in coronary blood flow, a marked decrease in renal, cutaneous, skeletal muscular and cerebral blood flows and no change of estimated splanchnic blood flow (7). From these circulatory responses, it is apparent that coronary and hepatic blood flow are maintained or increased under the influence of l-norepinephrine, while flow in other vascular circuits is decreased. Current experimental evidence places the blame for the development of irreversible surgical shock on failure of the liver and heart. If l-norepinephrine given to the shocked animal also produces relative increases of flow through these two vulnerable, vital organs, therein may lie a rationale for its use other than its vasoconstrictor action.

METHODS

Eighteen fasting, mongrel dogs (mean weight 10.6 Kg.) were anesthetized with morphine tartrate, 3 mg. per Kg. intramuscularly, followed in 30 minutes by chloralose, 48 mg. per Kg., and urethane, 480 mg. per Kg. intravenously. The right brachial artery, both femoral arteries, a femoral vein and a jugular vein were exposed and cannulated. Arterial pressure was measured with a capacitance type electromanometer and recorded on a multi-channel oscillograph. Mean arterial pressure was obtained by electrical integration of the electromanometric output. Oxygen was administered by intra-tracheal catheter. Heparin was used as an anticoagulant. Cardiac output was determined by the T-1824 Blue dye injection method. based on the Stewart principle (8). The dye concentration curve was measured with a calibrated dye photometer (9), the output of which was recorded on the oscillograph. Surface area was calculated from the nomogram of Cowgill and Drabkin (10). The base line for arterial pressure was taken at the skin of the back of the dog.

Experimental hemorrhagic shock was produced by the two-stage bleeding technique described by Wiggers (11). After the animals had been anesthetized and all apparatus was in satisfactory working order, two determinations of cardiac output, pulse rate, central venous and arterial pressures were made in close sequence. L-norepinephrine [4] was then administered intravenously by a constant infusion pump for ten minutes after which time the same determinations were repeated. (This series of determinations constitute the control period.) The l-norepinephrine infusion was then discontinued. After arterial pressure had returned to the base line level, bleeding was begun by syringe at a rate of 50 ml. per min. and continued until a pressure of 50 mm. Hg was reached. This level was maintained for several minutes. Another set of deter-

[1] This work is not to be construed as necessarily reflecting the views of the Department of the Navy.

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[3] CDR MSC USN.

[4] Levophed, 0.1 per cent, Winthrop-Stearns, New York 18, N. Y.

TABLE I

Effect of l-norepinephrine on mean arterial pressure *

Experiment	Body surface	Control pre inf.	Control inf. l-n'epi.	Hemorrhage first stage Early pre inf.	Hemorrhage first stage Early inf. l-n'epi.	Hemorrhage first stage Late pre inf.	Hemorrhage first stage Late inf. l-n'epi.	Hemorrhage second stage Early pre inf.	Hemorrhage second stage Early inf. l-n'epi.	Hemorrhage second stage Late pre inf.	Hemorrhage second stage Late inf. l-n'epi.	Return pre inf.	Return inf. l-n'epi.	L-norepi. infusion
	M.²	*mm. Hg*		*mm. Hg*		*mm. Hg*		*mm. Hg*		*mm. Hg*		*mm. Hg*		*μ/Kg./min.*
1	.51	89	91	52	54	61	58	36	52	37	48	—	—	.42
2	.41	89	99	54	79	61	73	40	58	47	59	—	—	.65
3	.45	116	124	47	55	47	53	35	58	35	46	—	—	.45
4	.53	84	94	56	87	56	70	27	78	34	22	—	—	—
5	.47	90	93	48	56	42	51	24	38	16	12	—	—	.51
6	.51	89	99	65	89	36	28	32	51	—	—	77	74	.24
7	.62	102	101	52	66	48	49	42	62	36	37	70	72	.47
8	.59	72	87	51	67	53	61	45	51	39	66	114	129	.47
9	.57	97	95	44	69	55	64	30	72	33	61	—	—	.55
10	.55	81	100	53	70	46	64	27	36	—	—	—	—	.50
11	.57	67	97	54	75	50	73	39	60	31	29	—	—	1.06
12	.61	98	110	50	70	43	46	29	43	29	37	119	125	1.03
13	.63	77	104	41	82	38	75	38	65	37	74	105	124	1.10
14	.64	70	89	53	76	49	63	32	41	28	25	104	106	1.00
15	.70	84	94	48	79	45	58	31	51	19	20	96	134	.84
16	.65	84	120	58	81	37	58	27	25	25	24	—	—	.98
17	.39	—	—	40	65	45	49	32	40	29	34	77	89	1.06
18	.43	69	79	46	87	67	86	31	45	34	29	89	99	1.07
M	—	86	98	51	73	49	60	33	51	32	40	95	106	.75
S.D.		12.55	10.45	5.99	10.63	8.60	12.69	5.56	13.05	6.72	18.30	16.40	22.80	
t		2.98		7.00		2.78		4.90		1.59		.96		
p		.0015		.0000		.0024		.0000		.055		.17		

* All figures are the means of two determinations.

TABLE II

Effect of l-norepinephrine on cardiac output *

Experiment	Control pre inf.	Control inf. l-n'epi.	Hemorrhage first stage Early pre inf.	Hemorrhage first stage Early inf. l-n'epi.	Hemorrhage first stage Late pre inf.	Hemorrhage first stage Late inf. l-n'epi.	Hemorrhage second stage Early pre inf.	Hemorrhage second stage Early inf. l-n'epi.	Hemorrhage second stage Late pre inf.	Hemorrhage second stage Late inf. l-n'epi.	Return pre inf.	Return inf. l-n'epi.
	ml./M.²/min.		*ml./M.²/min.*		*ml./M.²/min.*		*ml./M.²/min.*		*ml./M.²/min.*		*ml./M.²/min.*	
1	2261	1669	888	1050	1110	1440	372	480	378	404	—	—
2	1914	1710	972	1500	822	1038	630	678	618	702	—	—
3	1490	2615	798	1350	720	1038	450	972	552	858	—	—
4	1920	1991	858	1242	792	1301	558	1031	678	438	—	—
5	2051	2345	372	420	342	582	270	438	240	282	—	—
6	2430	2831	510	738	198	222	198	390	—	—	752	612
7	1595	2150	960	1271	828	990	648	960	540	480	2190	2290
8	1380	1500	858	1068	1029	1092	708	912	528	732	1631	1691
9	1781	1680	552	822	738	858	450	750	342	612	—	—
10	1980	2560	882	1151	822	1271	792	942	—	—	—	—
11	2021	1860	1217	1721	1880	1680	570	702	404	390	—	—
12	4140	4554	1068	1721	1152	1481	540	792	432	660	1961	1320
13	2261	1638	900	1320	900	1350	582	738	570	738	1519	1301
14	3370†	6600†	1050	1451	1490	2310	930	1145	404	586	1961	1680
15	1638	1780	1098	1700	942	1410	972	1580	672	708	3960	2940
16	1841	1511	786	1271	792	1211	492	552	420	318	—	—
17	—	—	492	696	414	498	324	404	192	210	930	972
18	1212	942	204	510	390	390	132	168	114	138	522	462
M	1994	2088	804	1170	852	1120	534	756	438	516	1716	1475
S.D.	662	821	271	412	406	482	230	334	165	212	1025	790
t	.35		3.14		1.75		2.26		.75		.51	
p	.36		.0008		.040		.012		.22		.30	

* All figures are the means of two determinations.
† Not included in statistical analysis.

minations was then made before and during the l-norepinephrine infusion which was given at the same rate and for the same time as during the control period (early first stage of hemorrhage). Arterial pressure was held at 50 mm. Hg for 75 minutes and measurements were made before and during the l-norepinephrine infusion as above (late first stage of hemorrhage). The animals were next bled at 50 ml. per min. to a pressure of 30 mm. Hg. Measurements were again made before and during l-norepinephrine as described above (early second stage of hemorrhage). Pressure was maintained at 30 mm. Hg for 75 minutes and the sequence of determinations was then repeated (late second stage of hemorrhage). All blood was next reinfused intravenously, blood pressure allowed time to stabilize, and the determinations made once again before and during the l-norepinephrine infusion (return period). In all experiments, the l-norepinephrine infusion was maintained at the same rate from beginning to end of the experiment regardless of the condition of the animal. No blood was administered to the animals during the determinations. The blood withdrawn for cardiac output determinations was replaced immediately after each pair of determinations had been completed.[5]

[5] Cardiac output determinations were made in pairs and the data presented in the tables are the means of each pair. Statistical analysis of the difference between all paired samples at all stages of hemorrhage gives the following results: mean of the first determination 645 ml. per min.; S.D. ± 512. Mean of the second determination 630 ml. per min.; S.D. ± 548. The difference between the means is not significant ($t = .276$, $p = .40$).

RESULTS

Data from eighteen experiments are presented in Tables I, II, and III. Figure 1 is a typical recording of the T-1824 dye concentration curves, right auricular, and femoral arterial pressures obtained from an animal in the early first stage of hemorrhage before and during the infusion of l-norepinephrine. In ten experiments, an average l-norepinephrine infusion of 0.47 μ per Kg. per min. was given and in eight 1.02 μ per Kg. per min. No significant difference is apparent when these two groups are treated separately.

In the control period l-norepinephrine produced the expected increase in mean arterial pressure ($t = 2.98$, $p = .0015$). However, the response of cardiac output to the drug during this period varied. At times it increased, sometimes it de-

TABLE III

Effect of l-norepinephrine on total peripheral resistance *

Experiment	Control		Hemorrhage first stage				Hemorrhage second stage				Return	
			Early		Late		Early		Late			
	pre inf.	inf. l-n'epi.	pre inf.	inf. l-n'epi.	pre inf.	inf. l-n'epi.	pre inf.	inf. l-n'epi.	pre inf.	inf. l-n'epi.	pre inf.	inf. l-n'epi.
	PRU†		*PRU*		*PRU*		*PRU*		*PRU*		*PRU*	
1	.0393	.0545	.0585	.0513	.0550	.0403	.0968	.1083	.0979	.1193	—	—
2	.0458	.0578	.0555	.0527	.0741	.0703	.0635	.0855	.0760	.0840	—	—
3	.0778	.0474	.0588	.0407	.0653	.0510	.0778	.0598	.0635	.0537	—	—
4	.0438	.0472	.0652	.0700	.0707	.0537	.0483	.0755	.0502	.0502	—	—
5	.0438	.0397	.1290	.1333	.1228	.0877	.0890	.0868	.0667	.0425	—	—
6	.0367	.0350	.1375	.1207	.1817	.1260	.1617	.1308	—	—	.1010	.1208
7	.0640	.0470	.0535	.0518	.0580	.0495	.0648	.0647	.0667	.0772	.0321	.0315
8	.0522	.0580	.0595	.0627	.0510	.0558	.0635	.0560	.0738	.0902	.0699	.0763
9	.0545	.0565	.0797	.0837	.0745	.0746	.0668	.0960	.0965	.0997	—	—
10	.0409	.0391	.0600	.0607	.0560	.0503	.0340	.0381	—	—	—	—
11	.0332	.0521	.0443	.0435	.0267	.0433	.0683	.0855	.0770	.0743	—	—
12	.0236	.0242	.0469	.0407	.0373	.0310	.0537	.0543	.0672	.0560	.0607	.0947
13	.0340	.0634	.0455	.0621	.0423	.0555	.0653	.0867	.0648	.1003	.0691	.0951
14	.0208‡	.0135‡	.0505	.0523	.0329	.0273	.0344	.0358	.0692	.0425	.0530	.0635
15	.0513	.0527	.0437	.0465	.0478	.0412	.0318	.0324	.0283	.0283	.0241	.0455
16	.0455	.0793	.0736	.0637	.0467	.0478	.0548	.0453	.0595	.0755	—	—
17	—	—	.0813	.0933	.1087	.0983	.0988	.0990	.1510	.1618	.0831	.0915
18	.0570	.0835	.2217	.1700	.1720	.2200	.2348	.2678	.2983	.2102	.1701	.2141
M	.0467	.0523	.0752	.0722	.0725	.0680	.0782	.0838	.0879	.0854	.0737	.0925
S.D.	.0193	.0150	.0434	.0350	.0443	.0448	.0492	.0532	.0622	.0471	.0434	.0532
t	.88		.22		.36		.32		.20		.77	
p	.20		.42		.36		.38		.42		.24	

* All figures are the means of two determinations.

† $PRU = \frac{1 \text{ mm. Hg}}{1 \text{ ml./min.}}$.

‡ Not included in statistical analysis.

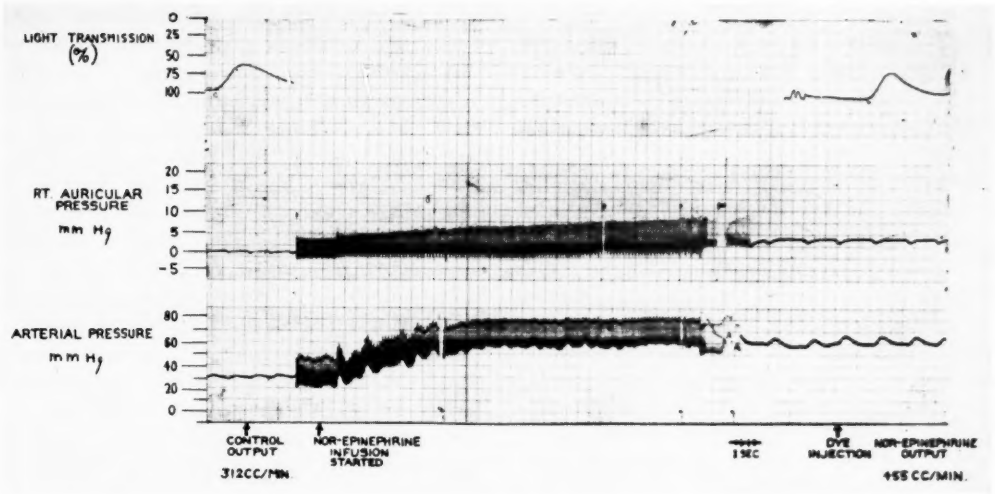

FIG. 1. THE EFFECT OF INTRAVENOUS L-NOREPINEPHRINE ON CARDIAC OUTPUT, RIGHT AURICULAR PRESSURE, AND ARTERIAL PRESSURE IN THE EARLY FIRST STAGE OF HEMORRHAGE (EXPT. NO. 9) PRIOR TO THE DEVELOPMENT OF IRREVERSIBILITY

Note the decrease in area under the T-1824 dye curve, which is indicative of an increase in cardiac output, during the infusion of l-norepinephrine. The apparent increase of right auricular pressure is due to shifting of the base line which is set at 9 mm. Hg. The rise of arterial pressure is pronounced (32 mm. Hg).

creased and sometimes it showed no change. This inconsistent response of cardiac output to l-norepinephrine was reflected in the total peripheral resistance which also showed a wide variation. In short, no consistent hemodynamic pattern could be discerned during the control period other than the increase of mean arterial pressure.

Significant increases of cardiac output occurred during l-norepinephrine infusion, during the early first stage of hemorrhage ($t = 3.14$, $p = .001$), during the late first stage of hemorrhage ($t = 1.75$, $p = .040$) and during the early second stage of hemorrhage ($t = 2.26$, $p = .012$). During the latter three stages, l-norepinephrine caused an increase in cardiac output during 49 of 51 determinations. There was no significant change of cardiac output during the late second stage of hemorrhage ($t = .75$, $p = .22$) or during the return period ($t = .51$, $p = .30$).

L-norepinephrine also elicited significant increases in mean arterial pressure during the first stage of hemorrhage and the early second stage of hemorrhage. This rise of pressure was due to increases in both systolic and diastolic pressures. As in the case of cardiac output, there was no significant change in pressure due to the drug in the late second stage of hemorrhage or in the return period. In those instances in which the infusion of l-norepinephrine was accompanied by a decrease of arterial pressure, the animals were in the "take-up" period of hemorrhagic shock and arterial pressure was already failing prior to the infusion.

Although the mean total peripheral vascular resistance during the l-norepinephrine infusion rose in all periods observed (Table III), in no instance is the rise statistically significant.

During experiments 1 to 9, right atrial pressure was measured. In the control period l-norepinephrine caused a rise of mean right atrial pressure (2.8 mm. Hg to 4.1 mm. Hg), which is not significant ($p = .12$). There was no change of mean auricular pressure during the remaining stages of the experiment.

DISCUSSION

The increase in mean arterial pressure observed in response to l-norepinephrine during the control period has been widely reported (7). Other observers have also noted the variation of response of cardiac output to the drug (7, 12). In the

presently reported series of experiments the effect on cardiac output during the control period was not uniform, varying from a 28 per cent decrease to a 75 per cent increase. However, in most experiments there was little change of resting cardiac output.

Brewster, Isaacs, and Wainø-Andersen (12) and Zanetti and Opdyke (13) have demonstrated that the response of both central and peripheral circulations to pressure amines is influenced to a great extent by the vasomotor status of the peripheral vascular bed prior to administration of the drug. Thus, comparison of data obtained from anesthetized dogs with that from alert human beings is not entirely justified. Others (14) have also stressed that the response of the circulation to different anesthetics may vary greatly. Brewster, Isaacs, and Wainø-Andersen (12) found that l-norepinephrine may effect a marked increase of cardiac output in the anesthetized dog. These facts probably account for the variations in response of cardiac output and peripheral resistance seen during the control periods of the present experiments.

The increase in cardiac output produced by the infusion of l-norepinephrine during the early stages of hemorrhagic shock was due to an increased stroke volume, since no tachycardia was observed. Two possible explanations for such an increased stroke volume may be offered: first, a direct central circulatory effect due to the direction stimulation of the myocardium by l-norepinephrine; second, an increase of venous return due to a peripheral circulatory effect of the drug. No effort has been made to assess the possible role of direct myocardial stimulation.

An increase in venous return may be explained by an increase in the effective circulating blood volume. In the shocked animal, a significant portion of the blood volume may lie stagnant in the veins and venules not immediately available to the general circulation. Thus, any agent causing venoconstriction may make a greater amount of blood available to the effective circulating volume. L-norepinephrine is a venoconstrictor and constricts the vessel of the spanchnic area as well as those of the muscle and skin (7). Hence, in response to the drug, the intravascular volume of the venous bed may be reduced and more blood made available to the effective circulation. These two factors would, in turn, increase venous return and, therefore, cardiac output.

A preparation has recently been devised with which changes of venous return and of available circulating blood volume may be demonstrated (15). Blood is diverted from the inferior and superior venae cavae into a reservoir from which blood is returned to the right heart. In this preparation (in the dog) injections of l-norepinephrine caused an increase of venous return and eight per cent sustained increment of blood in the reservoir.

During the irreversible stage of shock, in which l-norepinephrine was not effective in increasing cardiac output, venoconstriction was probably at a minimum due to a complete loss of venous responsiveness. If l-norepinephrine does play a direct role in stimulating the heart and effecting an increase of cardiac output, it is perhaps due to an increased coronary flow (16) and hence improved myocardial metabolism.

The preparation employed results in 82 per cent mortality after the end of the second stage of hemorrhage, and death within six hours after the reinfusion of the total volume shed (11). Therefore, the failure to demonstrate significant increases in cardiac output and arterial pressure during the late two stages of shock can be attributed to the condition of the animals at this time.

The increase of peripheral resistance occurring after hemorrhage is a well-known phenomenon. Remington and his associates have shown that Dibenamine® (Smith, Kline, and French), an adrenergic blocking agent, increases the survival rate of dogs in hemorrhagic shock presumably by preventing vasoconstriction which they believe may be a precipitating factor in irreversibility (17). However, in their experiments the bleeding volume was less than in the unmedicated animals. From these experiments one might conclude that vasoconstrictor agents such as l-norepinephrine are contraindicated in hemorrhagic hypotension. From the results of the present experiments, it would seem that such a conclusion may be erroneous, since l-norepinephrine effected no significant increase in peripheral resistance in any of the stages of hemorrhage.

Possible reasons for the lack of rise in peripheral resistance during the control are discussed

above. In the earlier stages of hemorrhage, the pressure rise effected by l-norepinephrine was accompanied by elevations of cardiac output. Late in hemorrhage neither cardiac output nor pressure increased.

The validity of the single dye injection method for determining cardiac output during hemorrhage in the dog is discussed by Hamilton and Remington (18). Others have compared this method with the direct Fick technique over a wide range of conditions and outputs in man (19).

The results of these experiments complement clinical observations of the effect of l-norepinephrine in oligemic shock. During the early stages of hemorrhagic hypotension when blood volume has not been critically depleted, and during severe hemorrhage prior to the development of irreversibility, the drug effected significant increases in cardiac output and arterial pressure. Moreover, during the experiments, the drug caused no adverse effects. After near fatal exsanguination or peripheral vascular failure, however, l-norepinephrine was of little therapeutic value. The above results would seem to justify the further clinical investigation of the effects of l-norepinephrine in the treatment of oligemic shock pending, or as an adjunct to, whole blood transfusion. The temporary relief afforded by the drug during hemorrhagic hypotension may have significant applications in the treatment of surgical cases provided it is clearly realized that whole blood transfusion is still the primary therapy of oligemic shock.

SUMMARY AND CONCLUSIONS

In experimental hemorrhagic hypotension in dogs, l-norepinephrine (0.75 μ per min. per Kg.) elicits significant increases of cardiac output and arterial pressure. Later in hemorrhage and after the development of irreversible shock, l-norepinephrine effects no significant change of arterial pressure or cardiac output. During no stage of hemorrhagic hypotension did the drug elicit an increase of total peripheral resistance.

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THE HEMODYNAMIC ALTERATIONS PRODUCED BY A PLASTIC VALVULAR PROSTHESIS FOR SEVERE AORTIC INSUFFICIENCY IN MAN [1, 2]

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Until recently, patients with aortic insufficiency and congestive heart failure have been offered only the temporary palliation provided by rest, diet, cardiac glycosides, and diuretics. A more direct therapeutic approach would aim at correction of the basic hemodynamic defect, by mechanical reduction of the volume of regurgitation.

Hufnagel (1) has devised a surgical procedure for altering the abnormal pattern of blood flow in aortic insufficiency. A plastic ball valve is inserted in the thoracic aorta, immediately distal to the left subclavian artery. This prosthesis prevents regurgitation of the blood traversing it, estimated to be approximately 75 per cent of the total cardiac output (2) (Figure 1). Blood flow above the valve, in the coronary, carotid, and subclavian systems remains regurgitant.

To date, over twenty-five patients have been treated successfully in this manner (3, 4). Clinical observations suggest that patients with severe aortic insufficiency and varying degrees of cardiac decompensation benefit markedly by this operation, showing postoperatively increased exercise tolerance and clearing of the signs of heart failure. The present study was undertaken in order to demonstrate objectively the alterations in circulatory dynamics produced by the valvular prosthesis.

MATERIAL AND METHODS

Nine patients (see Appendix) with severe aortic insufficiency as an isolated valve lesion were studied preoperatively and from 14 to 36 days postoperatively. All preoperative studies were carried out after several weeks of medical treatment in the hospital. The procedure of study was as follows:

Each patient fasted from midnight. Approximately 20 minutes before being brought to the laboratory they received 200 mg. sodium pentobarbital (Nembutal) orally.

Using local procaine anesthesia, a Cournand catheter was passed to the right heart under direct fluoroscopic control. The zero level for all right heart pressures was 5 cm. posterior to the angle of Louis. After recording the pulmonary arterial pressure, the catheter was withdrawn to the cavity of the right ventricle and left in place. In one patient (L. F.), the catheter was withdrawn to the right auricle because of the occurrence of premature ventricular contractions. This was the only case in which an ectopic rhythm was observed. In another (H. S.), the catheter could not be passed to the right heart; hence, direct Fick cardiac outputs were not obtained in this case.

A 15 gauge Robb type needle 5 cm. in length (5) was inserted through procainized skin into the femoral artery. The ample bore of this needle permitted unrestricted flow and adequate sampling when obtaining dye concentration curves.

Cardiac output was determined by the direct Fick method. Inspired air volume was measured from a Tissot spirometer (filled with room air) over a three-minute period. Expired air samples were collected in Douglas bags and their oxygen content determined immediately by a Pauling oxygen analyzer.[3] Oxygen consumption was calculated from expired air oxygen concentration, room air oxygen concentration, and inspired air volume corrected for temperature and barometric pressure. During the second minute of the three-minute air sampling period, simultaneous blood samples were drawn slowly from the right heart and femoral artery into oiled syringes. These were capped with mercury, kept on ice, and within three hours were analyzed for arteriovenous oxygen differences in a quartz spectrophotometer [4] by the method of Hickam and Frayser (6).

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[2] Sponsored in part by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] Arnold O. Beckman Company, Inc., South Pasadena, California.

[4] Arnold O. Beckman Company, Inc., South Pasadena, California.

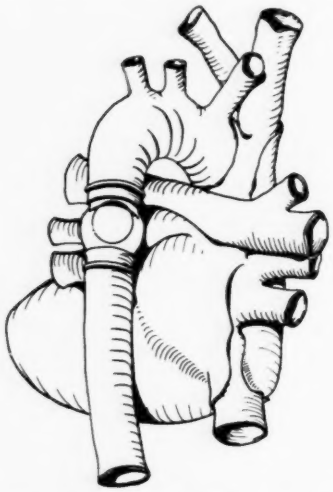

FIG. 1. SKETCH OF THE POSTERIOR ASPECT OF THE HEART AND GREAT VESSELS, SHOWING THE LOCATION OF THE PLASTIC AORTIC VALVE IN THE THORACIC AORTA, BEYOND THE LEFT SUBCLAVIAN ARTERY

An 18 gauge needle was then inserted in the brachial artery under local procaine anesthesia, and direct arterial pressure pulse contours were obtained. All pressures were recorded with strain gage manometers [5] using carrier-wave type strain gage amplifiers.[6] The strain gages were coupled directly to the large bore indwelling needles in both femoral and brachial arteries, with no intervening tubing. The frequency response curve of the direct-writing recorders used is flat to fifty cycles per second, and a 60 per cent response is obtained at 80 cycles per second. (In obtaining right heart pressures, a strain-gage was coupled directly to the hub of the indwelling catheter.)

The injection of T-1824 dye into the right heart, and arterial sampling were modified from the method of Werkö and his associates (7). The precise amount of dye delivered through each catheter into the right ventricle was determined by an *in vitro* injection. In every case, the same catheter used in the preoperative study was used in the postoperative study, and in each instance the approximate position of the catheter in the right heart was duplicated in the postoperative procedure. All blood samples were collected in paraffined tubes each containing a drop of one per cent heparin. In four cases, during the postoperative study, dye concentration curves were obtained from the brachial artery simultaneously with the femoral dye curves. The mean circulation times were obtained from the dye concentration curves by the method of Hamilton, Moore, Kinsman, and Spurling (8).

After obtaining the dye curve, additional arterial samples were obtained at 10, 15, and 20 minutes after the injection of dye, for determination of plasma volume (9). Total blood volume was calculated from the plasma volume and arterial hematocrit.

[5] Statham Laboratories, Beverly Hills, California.

[6] Sanborn Company, Cambridge, Massachusetts.

RESULTS

1. *Brachial and femoral arterial pressure pulse contours*

Tracings taken from the femoral artery postoperatively supplied evidence that the valve was

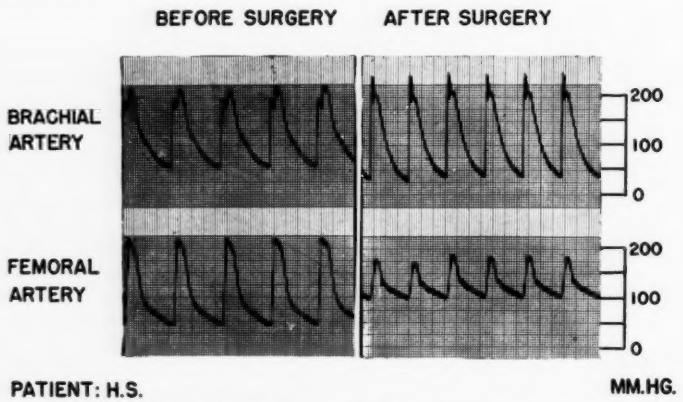

FIG. 2. CUTTINGS TAKEN FROM DIRECT RECORDINGS OF ARTERIAL PRESSURE PULSE CONTOURS IN THE BRACHIAL AND FEMORAL ARTERY IN PATIENT H. S. BEFORE AND AFTER SURGERY

While the wide regurgitant pressure pulse is transformed into a normal appearing contour in the femoral artery postoperatively, the brachial contour is widened due to an increased systolic pressure and a decreased diastolic pressure.

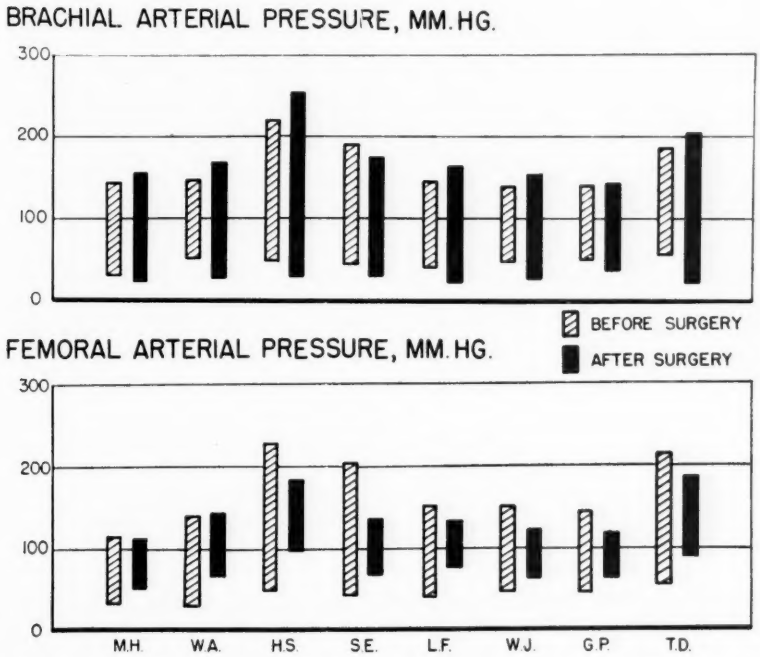

FIG. 3. CHART SUMMARIZING THE ARTERIAL PRESSURE CHANGES PRODUCED BY THE PLASTIC VALVE IN EIGHT CASES

In each instance the femoral pressures are altered towards normal, while the pulse pressure in the brachial artery is widened. In patient M. H., the preoperative brachial pulse pressure is slightly wider than the preoperative femoral pulse pressure. At operation a slightly coarcted area was found in the descending thoracic aorta which did not interfere with normal aortic blood flow. No collateral channels had developed, and this segment was left intact, the prosthesis being placed proximal to it.

functioning effectively. In each instance, the pressure pulse contour characteristic of free aortic regurgitation before operation was replaced after operation by a contour which appeared normal (Figures 2 and 3). Whereas the femoral arterial pulse pressure decreased toward normal, the brachial arterial pulse pressure widened postoperatively, due to both a decrease in diastolic pressure and (with one exception, S. E.) an increase in systolic pressure.

The configuration of the postoperative femoral arterial pressure pulse was of interest in that in most cases, a peculiar and variable notching occurred, perhaps due to closure of the rigid ball valve (Figure 4).

2. Cardiac output and stroke volume

In six cases, the cardiac index increased postoperatively by from 19 to 77 per cent of the preoperative values (Table I). The two exceptions were S. E., who exhibited no significant change, and T. D., who showed a decrease of 34 per cent in cardiac output. In S. E., the preoperative values may have been falsely high because of evident apprehension during the experimental procedure. For a similar reason the preoperative cardiac output in patient T. D., may not represent a basal value. His cardiac index preoperatively was the highest recorded in the eight cases studied, and the difference in the pre and postoperative cardiac output determinations lay entirely in the oxygen consumption, the arteriovenous oxygen difference actually decreasing postoperatively.

An increase in cardiac output usually was associated with both increase in oxygen consumption and a decrease in A-V oxygen difference. The exception to this was L. F., who exhibited a fall in oxygen consumption postoperatively, al-

TABLE 1

Data obtained pre- and postoperatively in nine patients with a plastic valvular prosthesis for severe aortic insufficiency exclusive of systemic arterial pressures

Patient	Days*	Body surface	O_2 consumption	A-V O_2 difference	Fick cardiac output	Average cardiac output	Per cent increase cardiac output	Average cardiac index	Per cent increase cardiac index	Heart rate	Effective stroke volume	Mean circulation time	Arterial hematocrit	Total blood volume	Pressures	
								L./min./M.² body surface							Pulmonary artery	Right ventricle
		sq. meters	*cc. per minute*	*volume per cent*	*liters per min.*	*liters per min.*			postop	*per min.*	*cc.*	*seconds*	*%*	*liters*	*mm. Hg*	*mm. Hg*
M. H.	1	1.47	148	4.8	3.1	3.25		2.2		66	49	23.5	40	4.08		
	pre		153	4.5	3.4											
	20	1.47	196	3.4	5.8	5.75	77	3.9	77	90	64	16.5	32	3.75		26/0
	post		201	3.5	5.7											
W. A.	8	1.67	175	6.4	2.7	2.6		1.55		68	38	55.0†	39	2.73		50/5
	pre		171	7.0	2.5											
	36	1.64	202	4.7	4.3											
	post		166	5.1	3.3	3.8	47	2.30	48	75	51	40.0†	33	2.61		30/0
C. F.	4	1.89	257	6.3	4.1	3.85		2.0		95	40	28.0	43	4.06		20/0
	pre		163	4.6	3.6											
	14	1.80	250	6.3	4.0	4.4	14	2.4	19	92	48	19.0	35	4.34		20/0
	post		300	6.3	4.8											
H. S.	7	1.93								75		24.0	39	7.14		
	pre															
	18	1.88								90		20.5	36	6.47		
	post															
S. E.	3	1.85	272	5.8	4.7	4.8		2.6		90	53	25.0	37	3.60		55/8
	pre		250	5.1	4.9											
	21	1.72	242	5.4	4.5	4.4	−10	2.55	− 2	100	44	24.5	33	4.33		30/0
	post		244	5.7	4.3											
L. F.	1	1.98	292	7.7	3.8	3.7		1.85		100	37	48.5†	44		27/10	25/2
	pre		314	8.6	3.6											
	21	1.90	260	5.8	4.5	4.45	20	2.35	26	100	45	27.5	40	4.92	25/14	28/4
	post		262	5.9	4.4											
W. J.	3	1.83	209	5.7	3.7											
	pre		205	5.4	3.8	3.75		2.05		80	47	25.0	48	5.50		18/2
	28	1.68	232	4.9	4.8											
	post		225	4.5	5.0	4.9	31	2.90	42	80	61	20.5	32	4.76		20/0
G. P.	9	1.92	238	4.8	5.0	4.9		2.55		86	57	22.5	43	5.09		36/7
	pre		237	5.0	4.8											
	30	1.82	250	4.4	5.8	5.95	21	3.25	28	86	69	17.2	38	5.85		36/4
	post		250	4.1	6.1											
T. D.	26	1.88	288	5.7	5.1											
	pre		306	5.8	5.2	5.15		2.75		80	64		40	5.47	76/30	
	30	1.77	198	5.5	3.6	3.6	−42	2.05	−34	70	52		34	4.36		30/0
	post		200	5.6	3.6											

* pre = Number of days before operation that studies were performed.
post = Number of days after operation that studies were performed.
† Mean circulation time determined with difficulty because of a markedly abnormal dye concentration curve.

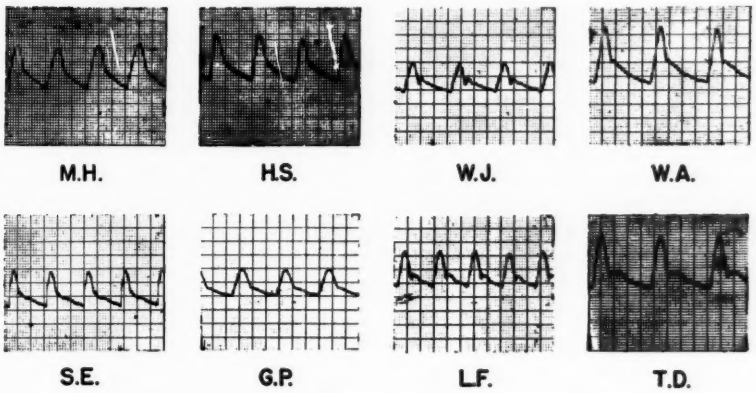

FIG. 4. CUTTINGS FROM THE POSTOPERATIVE FEMORAL ARTERIAL PRESSURE PULSE TRACINGS IN EIGHT PATIENTS

Although the general configuration of the pressure pulse is normal, there is a variable notching which is presumably due to closure of the rigid ball valve in the thoracic aorta.

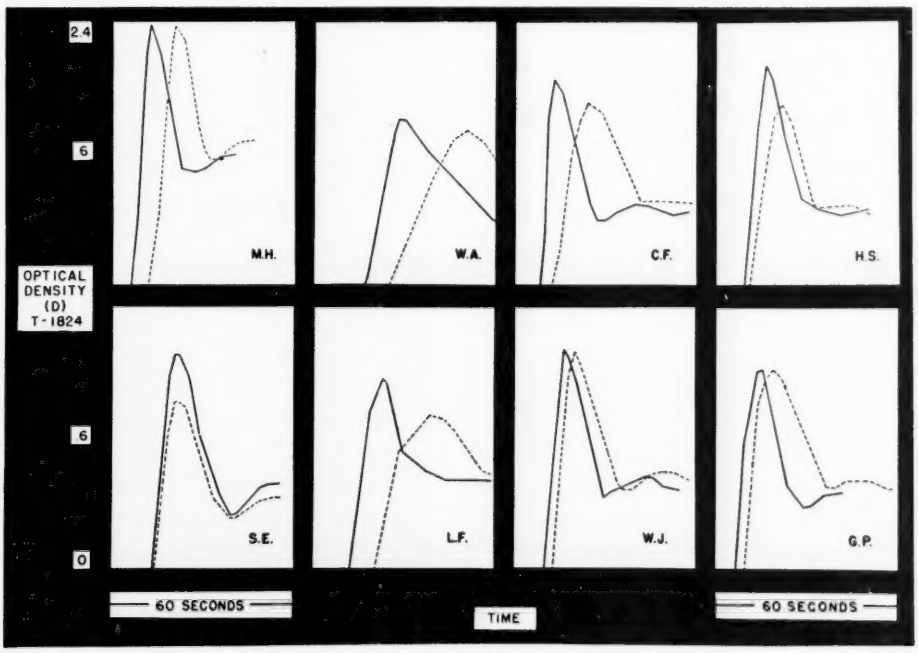

FIG. 5. PRE- AND POSTOPERATIVE FEMORAL T-1824 DYE CONCENTRATION CURVES IN EIGHT PATIENTS

The preoperative curves are the broken lines, the postoperative curves the solid lines. Each abscissa is a linear time scale from 0 to 60 seconds. Each ordinate is a logarithmic optical density scale from 0 to 2.4. With the exception of patient S. E., the postoperative femoral dye curve is steeper and more rapid than the preoperative curve. See text for discussion.

though there was a greater proportionate fall in A-V oxygen difference. In patient W. A., one of the increased postoperative cardiac outputs was due entirely to a decrease in A-V oxygen difference.

The effective stroke volume, calculated by dividing the average cardiac output by the heart rate recorded at the actual time of cardiac output measurement, usually increased paralleling the changes which occurred in cardiac output.

Certain discrepancies in the data were evident. One preoperative cardiac output value in patient C. F. was greater than one postoperative determination, although the average of the postoperative outputs was greater by 14 per cent. In all but one case, there was weight loss following operation. Weight loss was not due to diuresis, but rather the body tissue loss observed following major surgery, and in each instance had either ceased or reversed itself at the time of postoperative study. The decrease in body surface area postoperatively accounted for somewhat greater percentile increases in cardiac indices in the cases in which the cardiac output increased, and smaller percentile decreases in cardiac indices in the two cases in which the cardiac output decreased.

3. *Right heart pressures*

Of the seven cases in which right heart pressures were measured no change was observed in four while postoperative decreases were observed in three (W. A., S. E., and T. D., Table I). These were the only ones exhibiting elevations of right heart pressure preoperatively. In the case of T. D., the preoperative pulmonary artery pressure was 76/30 mm. Hg, and although the postoperative pressure was obtained in the right ventricle, it was significantly lower than the preoperative right heart pressure in the face of a marked decrease in postoperative cardiac output. S. E. showed no significant alteration in cardiac index, yet there was a postoperative fall in right heart pressure. These observations may be further evidence that the preoperative cardiac outputs in these two cases do not represent basal values.

4. *T-1824 dye concentration curves*

The alterations in shape of dye concentration curves obtained in the femoral artery in eight cases are shown in Figure 5. With one exception, the postoperative dye curve is steeper and more rapid than the preoperative curve. The exception is patient S. E., the only patient of this group who did not show a postoperative increase in cardiac output, or a postoperative increase in brachial artery systolic pressure. Mean circulation times calculated from these curves are summarized in Table I. The greatest reductions in mean circulation time occurred in the patients who showed the most abnormal preoperative values (W. A., L. F.).

In four cases postoperatively, dye transit curves were determined simultaneously in both the femoral and the brachial arteries (Figure 6). Despite the persistence of regurgitant flow above the valve, the alterations in the brachial dye curve

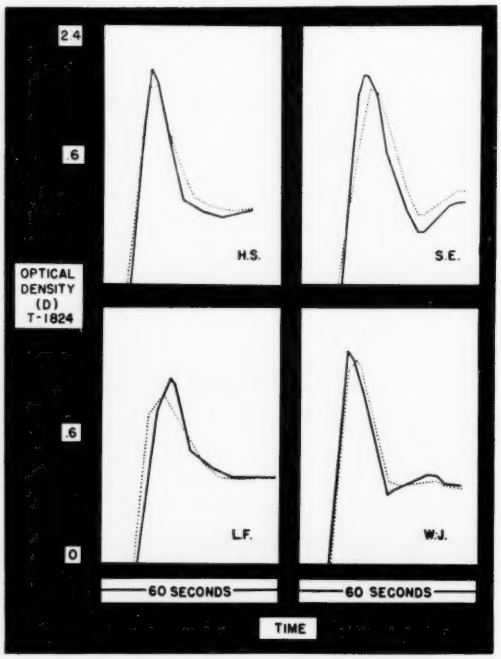

FIG. 6. SIMULTANEOUS POSTOPERATIVE DYE CONCENTRATION CURVES DRAWN IN BRACHIAL AND FEMORAL ARTERIES IN FOUR PATIENTS

Brachial curves are the dotted lines, femoral curves the solid lines. Reference to Figure 5 reveals that in each instance the alterations which occur in the shape and duration of postoperative femoral dye curves are simultaneously noted in the brachial dye curves. See text for discussion.

postoperatively corresponded to those in the femoral curve.

5. *Blood volume*

In all of the eight cases studied the arterial hematocrit was lower postoperatively. The reduction varied between 3 and 8 mm., except for patient W. J., whose hematocrit fell 16 mm. (Table I). Despite this constant finding there was no significant trend in total blood volumes, a slight postoperative rise occurring in three cases and a fall in five.

DISCUSSION

The cardiac output determinations and dye injection curves implied that the operation resulted in improved cardiac function. Cardiac output, cardiac index, and effective stroke volume were significantly increased in six of eight cases, postoperatively. Dye injection curves obtained from the brachial artery postoperatively showed a definite return toward normal in shape and duration corresponding to the alterations occurring in the femoral curves. Thus, the improvements in the femoral dye curves were not due to the correction of regurgitation, *per se*, since this improvement also was noted in the regurgitant brachial artery.

The source of improvement, therefore, seemed more likely to be cardiac in origin, possibly being secondary to more efficient ventricular contraction. This interpretation was supported by the cardiac output data. Those cases which exhibited an increase in cardiac output postoperatively also showed dye concentration curves of shorter duration, while the one patient with no change in cardiac output studied with dye injections failed to exhibit this dye curve alteration.

Considering the data as a whole, the following sequence of events seems to explain best the results observed: the introduction of the plastic valve reduced the absolute volume of regurgitation. Ventricular contraction then became more effective, permitting better emptying. This, in turn, resulted in increased cardiac output, decreased mean dye circulation time and other evidence of improved cardiac function.

Borden, Ebert, Wilson, and Wells (10) showed that in hypertensive heart and aortic valvular disease with left ventricular dilatation, prolonged mean dye circulation time (calculated, however, by a different method) correlated in part with increased diastolic volume of the heart. Gernandt and Nylin (11) had previously demonstrated the direct relationship of prolonged circulation time to the residual volume in the dilated left ventricle. It might be inferred, therefore, that decreases in mean circulation time in patients with left ventricular dilatation are due to decreases in the diastolic volume of their hearts. However, of the nine cases in this series only one (W. A.) showed a detectable diminution of diastolic heart size roentgenographically at the time that the postoperative studies were carried out, although longer term follow-ups revealed decreased heart size in all cases (4). This apparent inconsistency can be resolved by the explanation that a steeper dye curve of shorter duration may be due not only to a decrease in the diastolic volume of the ventricle, but also to a decrease in the residual volume of the ventricle *at the end of systole*. If the ventricle empties more completely with more vigorous contraction, there will be less pooling of blood in the ventricle to delay the passage of the dye.

Newman and his colleagues (12) believe that the character of the downslope of the dye injection curves obtained in the manner described is chiefly a function of pulmonary blood volume. The alterations in the postoperative dye curve observed in our patients might thus be attributed to a decrease in the volume of the pulmonary vascular bed. However, prior to surgery, intensive medical therapy had rendered our patients as free as possible of clinical congestive heart failure. The studies of Gernandt and Nylin (11) and of Borden, Ebert, Wilson, and Wells (10) indicate that the prolonged circulation time in heart failure depends on the amount of residual blood in the heart, and only to a slight extent on the degree of congestion. Hamilton recently has criticized the validity of the calculation of pulmonary blood volume from the slopes of dye curves (13).

The concept of more complete left ventricular emptying following operation also provides an explanation for the higher systolic and lower diastolic pressures in the regurgitant brachial artery, seen after as compared to before operation. More efficient systolic contraction would by increasing stroke volume raise the systolic pressure in the

brachial artery. More complete emptying would provide a greater pressure gradient from the regurgitant artery to the ventricle in early diastole, thereby lowering the brachial diastolic pressure.

SUMMARY AND CONCLUSIONS

1. The insertion of a plastic valvular prosthesis in the thoracic aorta, in nine patients with severe aortic insufficiency, successfully prevented regurgitant blood flow below the prosthesis.

2. In most instances, this resulted in improved cardiac function as evidenced chiefly by an increase in cardiac output and effective stroke volume, and a decrease in mean dye circulation time.

3. On the basis of the observed data, a possible sequence of events has been suggested as a mechanism by which more effective left ventricular contraction results from reduction of the absolute volume of regurgitation.

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APPENDIX

Brief Case Summaries

1. M. H., a 31 year old colored female, had acute rheumatic fever at age 5. At age 20 she experienced the onset of progressive dyspnea on exertion, orthopnea and paroxysmal nocturnal dyspnea for which she was digitalized. Two years prior to admission she developed angina pectoris which became progressively severe. There was marked enlargement of the left ventricle. She had the murmur of free aortic regurgitation with an Austin-Flint murmur and a ventricular diastolic gallop rhythm.

On September 11, 1952, she had a plastic valvular prosthesis inserted in the thoracic aorta, and a left thoracic sympathectomy. The postoperative course was uneventful and by two weeks following surgery there was a marked diminution of the intensity of the aortic diastolic murmur, the Austin-Flint murmur and the ventricular gallop rhythm. Thirteen months after operation she was well and working full time. She was taking no digitalis, but her requirements for nitroglycerin had not significantly decreased.

2. W. A. was a 50 year old white male with central nervous system and cardiovascular syphilis. Five years before operation he developed exertional angina, and three years later progressive and incapacitating dyspnea on exertion and ankle edema. He was treated with digitalis and frequent mercurial injections. In preparation for surgery, a diuresis of approximately 40 pounds was necessary. There was marked left ventricular enlargement. He had the murmur of aortic insufficiency, a prominent Austin-Flint murmur, and a loud ventricular diastolic gallop. His liver was palpable but there was no peripheral edema in the immediate preoperative period.

A plastic ball valve was inserted in the descending thoracic aorta on December 2, 1952, and a left thoracic sym-

pathectomy was performed. Postoperatively, the only complication was a transient azotemia with aggravation of previously existing paranoid tendencies. One month following operation there was a demonstrable decrease in heart size. Five months following operation he was completely asymptomatic and not taking digitalis. He did not adhere to his prescribed medical regimen. Nine months following operation he had paroxysmal auricular fibrillation, lapsed into frank congestive heart failure and developed marked azotemia. He died suddenly and autopsy disclosed multiple infarcts of mesentery, kidneys and lungs, and pyelonephritis. The plastic valve was intact.

3. C. F. was a 35 year old white male who had acute rheumatic fever at age 16. Three years prior to surgery he had auricular fibrillation converted with quinidine. Because of two subsequent attacks associated with marked dyspnea, he was placed on maintenance digitalis and quinidine. He had marked left ventricular hypertrophy. There was a murmur of free aortic insufficiency, an Austin-Flint murmur, and a ventricular diastolic gallop. Rhythm was normal sinus.

On January 12, 1953, he had a plastic valvular prosthesis inserted in the thoracic aorta. There were no postoperative complications. Three months following surgery he was working full-time, and there was a decrease in heart size on x-ray.

4. H. S., a 32 year old white male, had the diagnosis of aortic insufficiency of rheumatic origin made in 1945. There was no history of rheumatic fever or equivalents, and because of the presence of Marfan's syndrome, a congenital bicuspid valve damaged by endocarditis was also suspected. In 1948, he had subacute bacterial endocarditis, treated with penicillin. Three years prior to surgery, he experienced the onset of angina on exertion relieved by nitroglycerin. One year prior to admission he was digitalized and placed on salt restriction because of dyspnea on exertion and marked progression of his angina. Prior to surgery he was totally incapacitated by 20 to 30 anginal episodes daily precipitated by exertion or mental activity and often occurring at night. There were physical findings of free aortic regurgitation. The lungs were clear and there was no hepatomegaly or peripheral edema. Roentgenographically there was marked enlargement of the left ventricle.

A plastic valvular prosthesis was inserted in the thoracic aorta on February 6, 1953. Left thoracic sympathectomy was also performed. Although his postoperative course was complicated by a persistent pleural effusion, one month postoperatively there was a marked decrease in the patient's nitroglycerin requirements and a decrease in the intensity of his aortic diastolic murmur. At the time no decrease in the heart size was demonstrable by x-ray examination. He was well and working, requiring no nitroglycerin, when he suddenly expired nine months after operation. The valve was intact but other autopsy findings are not known at this time.

5. S. E., a 52 year old white male, had acute rheumatic fever at age 11. One and ½ years prior to operation he noted progressive dyspnea, on exertion, orthopnea and palpitation. He was treated with quinidine for a tachycardia the nature of which is unknown. He was digitalized but had taken digitalis only sporadically before entrance into the hospital for surgery. He had been unable to work for one year. There was no history of angina or ankle edema. He had the murmur of free aortic regurgitation, with an Austin-Flint murmur and a ventricular diastolic gallop rhythm. In addition, there was a faint systolic murmur in the apical region which was thought to possibly represent a mild degree of mitral insufficiency. The liver was palpable two centimeters below the right costal margin and nontender. There was no edema. A marked degree of left ventricular enlargement was evident by x-ray examination.

A valvular prosthesis was inserted in the thoracic aorta on March 30, 1953, and a left thoracic sympathectomy was performed. The postoperative course was uneventful, and by one month a definite decrease had occurred in heart size by x-ray examination. In two months the patient was taking no digitalis, and there was a marked diminution in both his aortic diastolic and Austin-Flint murmurs. The ventricular gallop was present but diminished. Nine months after operation he was working and apparently well.

6. L. F. was a 46 year old white male, the etiology of whose heart disease was in doubt. There was no history of rheumatic fever or equivalents. Two years prior to admission lues was diagnosed and treated with penicillin. One year prior to admission he noted the onset of progressive dyspnea on exertion and angina pectoris, requiring eventually 8 to 10 nitroglycerin tablets daily. He was not digitalized until one week prior to admission, at which time there was marked enlargement of the left ventricle and a slight degree of pulmonary congestion on the chest film. There were occasional premature ventricular contractions. Physical examination revealed the murmur of free aortic regurgitation with an Austin-Flint murmur and a ventricular diastolic gallop. There was no peripheral edema.

He had plastic aortic valvuloplasty on April 1, 1953, with left dorsal sympathectomy. Two weeks following operation the murmur of aortic insufficiency was less intense, as was the ventricular gallop, and the Austin-Flint murmur had disappeared. Twenty-five days postoperatively there was a detectable diminution in the heart size by x-ray examination. Nine months after operation he was working and apparently well.

7. W. J. was a 26 year old colored male who had acute rheumatic fever at age 13, with recurrence at age 14. For one year prior to operation he had had progressive dyspnea on exertion and occasional severe precordial pain for which he had not received nitroglycerin. He was digitalized prior to operation. There was the murmur of free aortic regurgitation with an Austin-Flint murmur and a ventricular diastolic gallop rhythm.

On April 13, 1953, he had a plastic aortic valvuloplasty and left thoracic sympathectomy. One month following operation the Austin-Flint murmur and the gallop rhythm had completely disappeared. The aortic diastolic murmur

had decreased several grades in intensity. Three months postoperatively he was on digitalis and was free of dyspnea and chest pain. There was a demonstrable decrease in heart size roentgenographically. Eight months following operation he died during the induction of spinal anesthesia for what was thought to be a surgical abdomen. Autopsy revealed acute gastroenteritis. The cardiovascular system was intact.

8. G. P. was a 21 year old colored male who had acute rheumatic fever in 1952. Just prior to his bout of acute rheumatic fever he had been rejected for the draft because of a heart murmur. One year prior to operation he had progressive dyspnea on exertion and an x-ray showed marked cardiomegaly. At that time he was digitalized. In addition to the murmur of severe aortic insufficiency and an Austin-Flint murmur, he had an aortic systolic murmur.

He had a plastic valvuloplasty on April 23, 1953, and a left thoracic sympathectomy. There were no postoperative complications. Two months postoperatively he was completely asymptomatic. There were no changes in the auscultatory findings in the heart, but there was a demonstrable decrease in heart size by x-ray examination. Four months following operation he died suddenly. It is known that at the time of death he was severely dyspneic and the valve was heard clicking at a rapid rate. No postmortem examination was obtained.

9. T. D. was a 40 year old colored male with a history of antiluetic therapy in 1939. In 1952 he noted rapidly progressive dyspnea with paroxysms at night. Six months prior to surgery there were two episodes of acute pulmonary edema. At the time of operation he had been digitalized for one year, and had required many injections of mercurial diuretics. He had a loud "cooing dove" type of aortic diastolic murmur, a normal sinus rhythm, and marked cardiomegaly with the roentgenographic appearance of aneurysmal dilatation of the left ventricle.

On April 17, 1953, a plastic valve was inserted in his thoracic aorta which was markedly thickened. A left thoracic sympathectomy was also performed. There were no postoperative complications. Although there has been no essential change in the aneurysmal configuration of the heart by x-ray, nine months following operation the patient was on a regular diet, receiving no digitalis or diuretics, and there were no signs or symptoms of congestive heart failure. He has been noted to have occasional bouts of frequent premature ventricular contractions which have not been treated.

ACKNOWLEDGMENTS

The authors wish to acknowledge the valuable technical assistance of Miss Jean Pietras. We are indebted to Messrs. Vic Landi and Larry Gaskins for the photographic reproduction of illustrations and charts.

URINARY EXCRETION OF NUCLEIC ACID DERIVATIVES IN PERSONS WITH MYELOID LEUKEMIA[1]

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Considerable evidence has been presented to link growth and maturation of granulocytic leukocytes with cellular nucleic acid metabolism. The mechanisms of this relationship, however, remain unclear. Thus, Thorell (1), by microspectrophotometric techniques, has correlated the concentration of ribonucleic acid (RNA) present in the cytoplasm and nucleolus with growth potentialities of the blood cell. He showed that during maturation, cellular RNA concentration decreased progressively, paralleling the declining growth activity of the cell. Furthermore, he demonstrated the failure of rapidly proliferating cells in acute leukemia to show the rapid decline in RNA metabolism which is seen in normally proliferating cells following their active growth phase.

These differences in RNA content between immature and mature blood cells have been confirmed by Davidson, Leslie, and White (2). In this study, peripheral blood cells showed a significantly lower content of RNA phosphorus than bone marrow cells both in normal and in leukemic persons. However, decrease in the content of desoxyribonucleic (DNA) phosphorus in peripheral blood cells as compared with marrow cells was of borderline significance.

Further evidence relating cell growth with nucleic acid metabolism is found in the clinical response of some of the leukemias to substances, including folic acid antagonists, nitrogen mustards, urethane, cortisone and arsenic, which have been shown to inhibit biological synthesis of nucleic acid (3, 4). Furthermore, clinical observations by Vilter and his associates (5) relate the aberrant erythropoiesis in megaloblastic anemias with abnormal nucleic acid metabolism conditioned by deficiences of Vitamin B_{12} and folic acid.

Abnormalities in nucleic acid metabolism associated with aberrant cell growth in the leukemic organism might be reflected in the urinary excretion of intermediary products derived from these metabolic processes. In the present study, chromatographic and ultraviolet spectroscopic techniques have led to the identification of ultraviolet absorbing substances in the urine of persons with myeloid leukemia which are not present in the urine of non-leukemic subjects. One of these substances has been identified as uracil, a pyrimidine component of RNA.

MATERIAL AND METHODS

Five consecutive adult patients with myeloid leukemia admitted to the medical service of the University Hospitals of Cleveland served as subjects in this study. The peripheral blood hemograms of these patients at the time of this study are shown in Table I. Four of these (J. S., B. W., E. W., and J. McK.) presented a clinical diagnosis of chronic myeloid leukemia with peripheral leukocytosis, splenomegaly, anemia, and granulocytic hyperplasia in the bone marrow. The fifth patient (R. N.) was diagnosed as having leukemia of a myelomonocytic type. Although the peripheral leukocyte count of this patient was not greatly increased, abnormally young forms were noted in stained blood films; and the bone marrow showed marked leukocytic hyperplasia with 53 per cent blast forms.

Three patients (J. McK., E. W., and R. N.) had received no previous therapy. Patient J. S., had received two courses of splenic irradiation 16 and 10 months prior to this study. Patient B. W., had undergone a splenectomy elsewhere six years previous to this study. She had received radioactive phosphorus one year previously when a diagnosis of chronic myeloid leukemia was established. At the time of this study, hepatomegaly and adenopathy were marked and bone marrow biopsy revealed moderate myelofibrosis.

In addition, four non-leukemic patients on the medical wards of the hospital were studied as controls.

In order to minimize the urinary excretion of substances with ultraviolet absorption at wave lengths characteristic of nucleic acid derivatives (260–290 millimicrons), all subjects in the study were placed on an animal-protein-free diet with restriction of coffee, tea, and

[1] This study was supported by a grant from the Montgomery County (Ohio) Society for Cancer Control and by the Price McKinney Fund.

TABLE I

Peripheral blood findings in patients with myeloid leukemia

Patient	J. S.	B. W.	E. W.	J. McK.	R. N.
Sex	M	F	M	M	F
R.B.C. (Millions)	2.64	2.74	2.49	3.34	2.64
Hgb. (Grams)	6.7	8.4	6.5	10.0	8.0
W.B.C.	440,000	131,000	318,000	85,000	13,000
% Neutrophils	42	57	51	47	54
Metamyelocytes	9.5	13	23	19	11
Myelocytes	29.5	5	15	11	12
Eosinophils	4.5	2	3	13	—
Basophils	5.5	4.5	2	2	1
Monocytes	0.5	7	2	4	10
Young Monocytes	—	1.5	—	—	7
Blasts	7.5	4.5	2	—	7
Lymphocytes	1	5.5	2	4	8
Normoblasts per 100 WBC	6	—	2	4	1

chocolate. After three days of this dietary restriction, 24-hour urine collections were made; three milliliters of concentrated hydrochloric acid were placed in each collecting bottle as a preservative. One one-hundredth of the total 24-hour urine volume was diluted tenfold with distilled water and the pH adjusted to 10 with concentrated ammonium hydroxide. Ultraviolet absorbing components in the urine were separated by collection of fractions in which absorption was increased according to the method of ion-exchange column chromatography described by Cohn (6). The diluted urine was passed through a column of anion exchange resin (Dowex-1, chloride form, bed size 12 cm. × 1.77 cm^2.). After washing to neutrality with water, the column was eluted with 0.003 N hydrochloric acid; this eluate was collected in 20 ml. fractions. The ultraviolet absorption of successive fractions was measured in a Beckman quartz spectrophotometer, Model DU, at a wave length of 260 millimicrons. These collections were then distilled to dryness *in vacuuo* at a temperature not exceeding 50°C. The residue was redissolved in distilled water, 1.0 ml.; 0.1 ml. of this solution was then spotted on a strip of

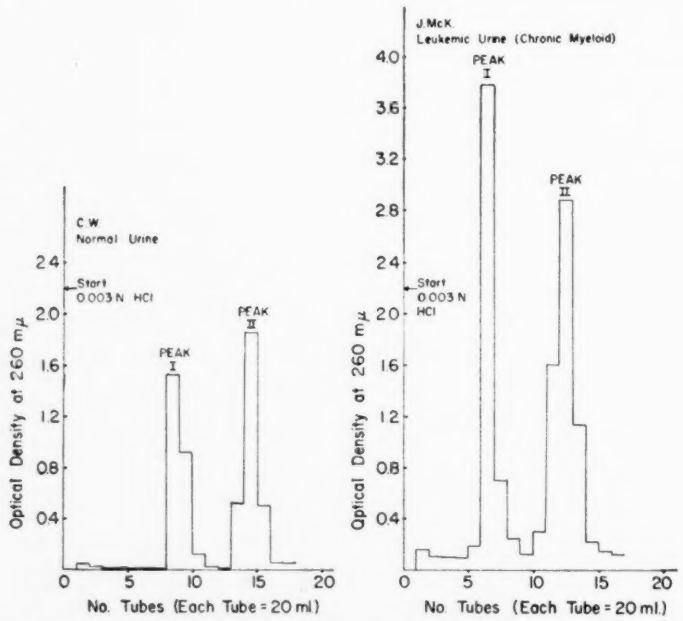

FIG. 1. ANION EXCHANGE SEPARATION OF SUBSTANCES IN NORMAL (1a) AND MYELOID LEUKEMIC (1b) URINE

Conditions for adsorption and elution are described in text.

Whatman No. 1 filter paper for paper chromatographic studies. Ascending chromatograms were developed overnight using a 5 per cent K_2HPO_4-isoamyl alcohol mixture as solvent as described by Carter (7). After drying in air at 60°C., component ultraviolet absorbing compounds were identified by exposure in the dark of the chromatogram to a source of ultraviolet light (Mineralight lamp, Model SL 2537). For spectral analysis, the spots were recovered from the chromatogram by cutting the areas showing absorption into small strips and eluting with water at 37°C. overnight.

RESULTS

Representative diagrams of ultraviolet absorbing components in normal and leukemic urine eluted from the anion exchange column are seen in Figures 1a and 1b, respectively. Comparison of these two elution graphs shows in each instance the presence of two absorbing peaks, both of which are greater in the leukemic than in non-leukemic urine. The increased absorption of the first peak was seen in all five of the leukemic specimens examined (Table II), ranging in the leukemic urine from 5900 to 11,260 and in the normals from 3700 to 5480 260 mμ absorption units [2] per 24 hours.

TABLE II

Quantitation of ultraviolet absorbing peaks recovered from anion exchange column chromatography of leukemic and non-leukemic urine

	Patient	Peak I Units* per 24 hrs. (260 mμ)	Peak II Units* per 24 hrs. (290 mμ)	Peak II Mg. uric acid† per 24 hrs.
Myeloid Leukemics	J. S.	7,280	26,620	458.9
	B. W.	8,160	44,930	774.6
	E. W.	11,260	55,720	960.6
	J. McK.	7,340	34,165	589.0
	R. N.	5,900	26,330	454.0
Normals	C. J.	5,480	24,180	416.8
	D. H.	4,950	20,140	347.2
	S. M.	3,700	9,300	160.3
	C. W.	4,900	13,360	230.3

* See footnote 2 to text.
† See text for method of converting absorption units to milligrams.

Paper chromatography of this fraction in normal urines showed the development of one ultraviolet absorbing component with an Rf value of 0.78 (Figure 2). This component was also present in leukemic urines and gave a positive qualitative test for phenols (8). Four ultraviolet fluorescent spots were also developed; these did not show significant absorption, however.

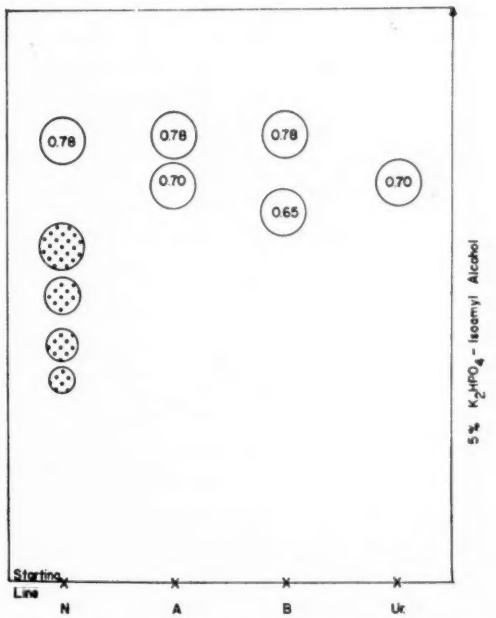

FIG. 2. DIAGRAMS OF REPRESENTATIVE PAPER CHROMATOGRAMS OF PEAK I URINE FRACTIONS

Rf values represented within each spot. N = Normal urine; A = Patients J. S., B. W., and R. N.; B = Patients J. McK., and E. W.; Ur = Uracil. Dotted areas represent fluorescent areas without significant absorption. Similar fluorescent spots are present in all chromatograms but are not included in the diagrams.

In paper chromatograms of this fraction from three of the leukemic urines examined (Figure 2A), an ultraviolet absorbing substance with an Rf value of 0.7 was seen; this compound was not present in normal urine. In all three instances, the ultraviolet spectrum of this component was the same (Figure 3a) and was similar to that of uracil (Figure 3b). Also, this compound was similar to uracil in its paper (Figure 2, Ur) and anion exchange chromatographic properties. Furthermore, this substance supported growth [3] for a yeast mutant *Saccharomyces cerevisiae*, reported by Pomper (9) to require uracil, uridine, uridylic acid, or 5 nitro uracil for growth. Of this group

[2] The number of absorption units was obtained by multiplying the volume of the effluent fraction by its optical density at the wave length specified. This value was then multiplied by 100 for the total 24 hour quantitation.

[3] The author is grateful to Dr. Irwin A. Rose, presently of the Department of Pharmacology, New York University, who performed these microbiological studies.

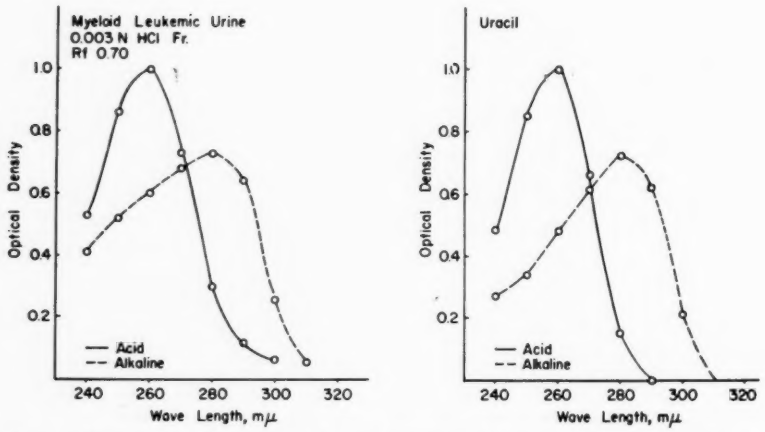

FIG. 3. ULTRAVIOLET SPECTRUM OF (a) SUBSTANCE, Rf VALUE OF 0.7, ISOLATED FROM URINE OF LEUKEMIC PATIENTS, J. S., B. W. AND R. N. AND OF (b) URACIL.

of substances, the spectral properties of this compound from leukemic urine identify it as uracil (10). Quantitation of the uracil excreted in the urine of two patients (R. N. and J. S.) showed a 24-hour excretion of 40.5 mg. in each patient (1.0 mg. uracil = 74 absorption units at 260 mμ).

In similar paper chromatograms of this first fraction of urine specimens from the other two leukemic patients (Figure 2B), uracil was not found. However, in each instance a component with an Rf value of 0.65 was observed which was not present in normal urine. The ultraviolet absorption spectrum of this component was the same in both specimens and is recorded in Figure 4. Further characterization of this component has not been accomplished. Nothing in the clinical evaluation of these patients was apparent to explain the presence of this different component in the urine of these two patients.

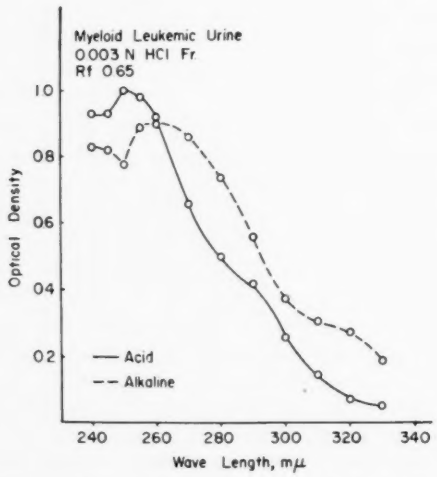

FIG. 4. ULTRAVIOLET SPECTRUM OF SUBSTANCE, Rf VALUE OF 0.65, ISOLATED FROM URINE OF LEUKEMIC PATIENTS J. MCK. AND E. W.

The second ultraviolet absorbing fraction of the elution diagrams (Peak II, Figure 1a and b) in all specimens demonstrated maximum absorption at 290 millimicrons and represents uric acid. From the extinction coefficient of uric acid at this wave length (1.0 mg. uric acid = 58 units at 290 mμ), the 24-hour excretion of uric acid can be quantitated. These values are shown in Table II. Under the conditions of this experiment, the uric acid excretion in the four control subjects varied from 160.3 to 416.8 mg. per 24-hour urine specimen. In the five leukemic patients, the urinary excretion of uric acid varied greatly from patient to patient ranging from 458.9 to 960.6 mg. per 24 hours. In two of these (E. W. and C. W.) the elevation was marked, 960.6 and 774.6 mg. per 24 hours, respectively. In one (J. McK.), the elevation was moderate, 589.0 mg. per 24 hours. In the remaining two (J. S. and R. N.), the values, 438.9 and 454 mg. per 24 hours, respectively, were not greatly increased above those obtained in some normals. Again, there was nothing apparent in the clinical evaluations of these patients to explain

these differences in uric acid excretion. Furthermore, the increased excretion of uric acid could not be correlated with the urinary excretion of uracil in this group of patients.

DISCUSSION

The presence of uracil in the urine of some persons with myeloid leukemia again brings attention to abnormalities in nucleic acid metabolism in this disease. Since uracil is a primidine component of RNA and not of DNA, this finding suggests the relationship of altered RNA metabolism to the abnormal growth and maturation of leukocytes in these patients.

The recent work of Caren and Morton (11) indicates that the metabolic fate of exogenous uracil in man involves rupture of the pyrimidine ring with excretion of most of its nitrogen as urea. However, work in this laboratory (12) has shown that a small amount of uracil is excreted as such when administered to normal persons. Thus, when two grams of uracil were given orally, about 126 mg. were recovered intact in the subsequent 24-hour urine collection.

The urinary excretion of uracil in some myeloid leukemics indicates its production endogenously in amounts which cannot be cleared by existing catabolic processes. Our present limited knowledge of nucleic acid metabolism in man, especially of the pyrimidines and their intermediates, permits only a speculative analysis concerning possible mechanisms for this increased endogenous uracil. The possibility exists that this is the reflection of increased destruction of immature cells, the RNA content of which is very high. However, the failure to recover thymine, a pyrimidine characteristic of DNA, seems against total cellular destruction as the mechanism. Furthermore, in two of the patients who excreted uracil (J. S. and R. N.), the excretion of uric acid did not reflect greatly excessive tissue destruction.

Other mechanisms which result in increased endogenous uracil might include (*1*) an extremely rapid turnover in RNA metabolism or (*2*) increased endogenous production of uracil which is not incorporated into cellular nucleic acid. The former explanation does not seem feasible in view of the high concentration of RNA present in leukemic cells (1, 2); and again, because of failure to demonstrate a proportional rise in uric acid excretion which would reflect increased breakdown of purine moieties of nucleic acid. Speculatively, the latter mechanism might imply some abnormality in the intermediary metabolism of pyrimidines. Further study is needed, however, for clarification of this as a possible metabolic aberration in these patients.

Increased endogenous uracil might also result from a deficiency in enzyme systems concerned in the catabolism of the pyrimidine ring to urea and oxalic acid. Uracil tolerance studies in normal and in leukemic persons now in progress in this laboratory may provide information concerning this as a possible mechanism.

The significance of the abnormal ultraviolet absorbing component recovered in patients J. McK. and E. W. awaits further characterization of this substance. However, its presence in two leukemic patients and its absence in normals suggests some relationship to the leukemic process in these individuals.

SUMMARY

1. Abnormal ultraviolet absorbing substances have been identified in the urine of five patients with myeloid leukemia.

2. In three of these patients, the pyrimidine uracil has been identified spectroscopically, chromatographically, and microbiologically.

3. In the other two patients, another substance, identified chromatographically and spectroscopically, was excreted; this substance has not been further characterized.

ACKNOWLEDGMENTS

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EFFECTS OF HEPARIN ON ALIMENTARY HYPERLIPEMIA. AN ELECTROPHORETIC STUDY [1]

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A few recent communications deal with electrophoretic observations concerning the effect of heparin on alimentary hyperlipemia. Nikkilä (1) found by moving boundary electrophoresis a decrease of beta-1 globulin and an increase in alpha-1 globulin after the injection of heparin. Rosenberg (2) and Fasoli (3) noted on paper electrophoresis an increase in the mobility of the beta lipoproteins which migrated in or near the alpha-2 globulin area. On the other hand, Spitzer (4) stated that administration of heparin does not alter the electrophoretic patterns of serum proteins in either fasting or lipemic dogs.

To obtain further information about the effect of heparin on the plasma proteins in alimentary hyperlipemia normal individuals were given a meal rich in fat and thereafter the electrophoretic pattern of the serum was studied both before and after the intravenous injection of heparin. Electrophoretic analysis was made by paper electrophoresis as well as by moving boundary electrophoresis. The results of these studies are described in this paper.

MATERIALS AND METHODS

Collection of blood. Ten ml. of blood were withdrawn from eight normal individuals in a fasting state, after which a meal was given consisting of one pint of heavy cream, 16 Gm. of cocoa and 32 Gm. of sugar. Four and one-half hours after the ingestion of the meal, another 10 ml. of blood were withdrawn and subsequently 50 mg. of heparin (Lederle) were injected intravenously. Fifteen minutes after the injection of heparin, 10 ml. of blood were taken again. The blood samples were allowed to clot at room temperature, then were centrifuged and the serum removed with a pipette.

Analytical methods. For paper electrophoresis the method of Grassmann and Hannig (5) was used. Serum in the amount of 0.02 ml. was placed on a paper strip (Whatman No. 1) and the electrophoresis chamber was filled with sodium barbital-sodium acetate buffer of pH 8.6 and ionic strength 0.1. Electrophoresis was carried out at 110 volts for a period of 12 hours in a room at constant temperature. In order to determine the relationship of the lipids to the protein fractions, two paper strips were used simultaneously in one chamber under identical experimental conditions. One paper strip was dried at 100° C. and stained with Amidoblack 10 B for detection of proteins. The other one was dried at 37° C. and stained with Sudan Black B according to the technique of Swahn (6) for the determination of lipids. Moving boundary electrophoresis was carried out in a Perkin-Elmer model 38 Tiselius electrophoresis apparatus, using a barbital-sodium citrate buffer of pH 8.6 and ionic strength 0.1. Prior to electrophoresis, the serum was dialyzed for 12 hours in this buffer.

The turbidity of the plasma samples was determined according to the method of Geyer, Mann, and Stare (7), except that the difference between the reading for the sample and the blank was divided by 10 and expressed in units.

RESULTS

Paper electrophoresis

In the fasting state two distinct bands were found on the paper strips stained for lipids. One intensely stained band was present in the area of the beta globulin, corresponding to the beta-lipoproteins; the other, less intensely stained, was in the area of the alpha-1 globulin, corresponding to the alpha-lipoproteins (Figure 1 A).

The samples of serum taken four and one-half hours after the ingestion of the fat meal showed a third and new lipid band due to the chylomicra, near the starting line where the hyperlipemic serum had been placed on the paper strip (Figure 1 B).

In the samples obtained fifteen minutes after the intravenous administration of 50 mg. of heparin the following changes were observed. The lipid material near the starting line had disappeared; the lipid band normally present in the beta globulin area then appeared in the area of alpha-2 or even alpha-1 globulin, and instead of the lipid band,

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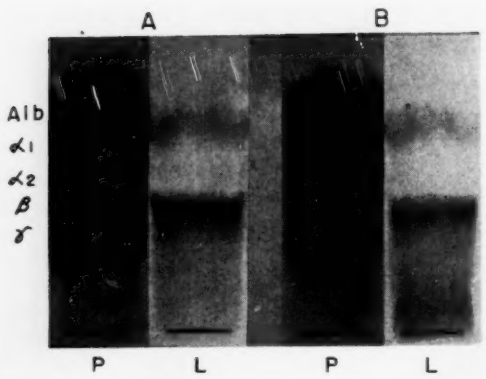

FIG. 1. PAPER ELECTROPHORETIC PATTERNS OF THE SERUM OF A NORMAL INDIVIDUAL IN A FASTING STATE (A) AND IN AN ALIMENTARY HYPERLIPEMIC STATE (B)

The paper strips marked P are stained for proteins, those marked L are stained for lipids. In the fasting state (A) on the lipid stained paper strip (L) an intensely stained band is present in the beta globulin area and a less intensely stained band in the alpha-1 globulin area. In the alimentary hyperlipemic state (B) a new band is present on the lipid stained paper strip (L) near the starting line where the serum was placed.

normally present in the alpha-1 globulin area, lipid material migrated ahead of the albumin (pre-albumin component) (Figure 2). The paper strips stained for proteins did not show any protein component ahead of the albumin. The relative values of the protein fractions of the sera taken during the fasting state, after the ingestion of fat and after the injection of heparin, showed no significant changes. The phenomena described above were observed in all eight cases studied.

Moving boundary electrophoresis

In the fasting state normal electrophoretic patterns were obtained in each of the eight cases. After the ingestion of the fat meal the serum became markedly turbid and it was therefore impossible to obtain satisfactory electrophoretic patterns in all eight cases. In the ascending pattern the albumin, alpha-1 and a part of the alpha-2 globulin peak were well visualized, while the rest of the pattern was rather faint and sometimes invisible. In the descending pattern the contrary occurred and the albumin, alpha-1 and a part of the alpha-2 globulin peak were faint. This suggests that the chylomicra, which contribute largely to the turbidity, migrated with the approximate mobility of alpha-2 globulin and scattered the incident light intensively, causing incomplete exposure of the photographic film. After the injection of heparin, concomitant with the decrease in turbidity, this phenomenon was either absent or present to a much lesser degree, so that distinct patterns were obtained.

Because the patterns were severely obscured in three cases, calculation of the relative values of the protein fractions in the hyperlipemic sera was possible only in five cases. In the five cases the average of the relative values for alpha-1, alpha-2, and beta-1 globulin were slightly increased as compared with the values obtained during the fasting state. The alpha-1 globulins increased from 6.7 to 7.9 per cent, the alpha-2 globulins from 8.3 to 9.4 per cent, and the beta-1 globulins from 13.2 to 14.0 per cent of the total proteins (Table I).

After the injection of heparin the electrophoretic pattern was changed in all eight cases. There was a marked decrease in the size of the beta-1 globulin peak together with an increase in the alpha-1 or alpha-2 globulin peak, or in both of them. In comparison with the fasting values, the beta-1 globulin was decreased from 13.2 to 8.6 per cent, and the alpha-2 globulin was increased from 8.3 to 9.9 per cent, and the alpha-1 globulin increased from 6.7 to 9.0 per cent of the total proteins (Table I).

In the serum withdrawn after the injection no pre-albumin peak was seen on the photograph taken after the usual 55 minutes of electrophoresis. This was in contrast to the observations made by paper electrophoresis where a distinct pre-albumin component was present. This discrepancy was explained by studying the developing electrophoretic pattern. A pre-albumin peak appeared after 10 minutes, remained in the visual field for a maximum of 40 minutes and was no longer visible at 55 minutes (Figure 3). The mobility of the pre-albumin component was calculated in one case giving a value of -9.8×10^{-5} cm^2./volt sec compared with a normal value of -6.0×10^{-5} cm^2./volt sec for albumin. The pre-albumin component could, however, be demonstrated after 55 minutes of electrophoresis. This only occurred if the protein solution in the U-cell was not pushed

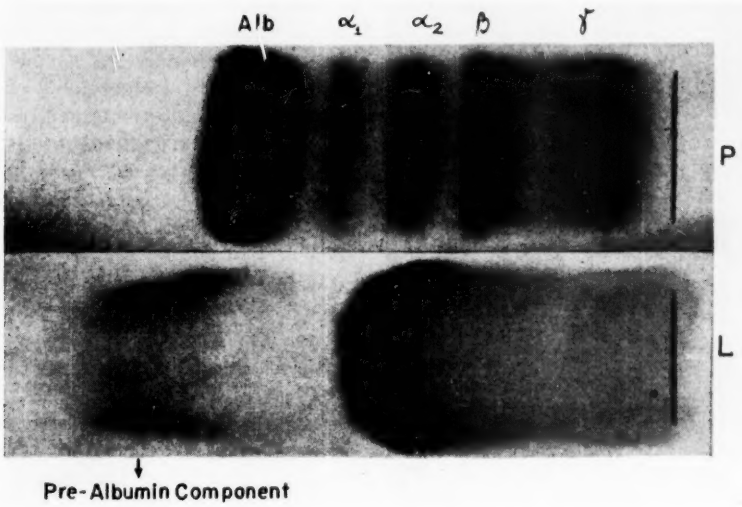

FIG. 2. PAPER ELECTROPHORETIC PATTERNS OF AN ALIMENTARY HYPERLIPEMIC SERUM AFTER INJECTION OF HEPARIN

P is stained for proteins, L is stained for lipids. On the lipid stained paper strip, a pre-albumin component can be seen and another lipid material in the alpha-2 globulin area.

down by addition of buffer to make a visible boundary, as is usually done at the beginning of an electrophoretic experiment. In that case the protein fractions had to migrate a longer distance and, after 55 minutes, a pattern was obtained showing the various protein fractions including the pre-albumin peak.

The relative concentration of the pre-albumin component was calculated from the electrophoretic patterns after 30 minutes. The values ranged from 3.4 to 7.9 per cent, averaging 5.6 per cent of the total concentration of the serum proteins.

Turbidity

The average turbidity values of the plasma increased after the ingestion of fat from the normal 3.0 units to 11.3 units, and decreased to 7.1 units fifteen minutes after the injection of heparin.

Controls

Control observations were made on five of the same persons, used in our studies. Heparin in the amount of 50 mg. was injected intravenously during the fasting state. In the paper electrophoresis no changes were found in the distribution of the

TABLE I

Average values of the serum protein fractions obtained on eight normal individuals by moving boundary electrophoresis *

State of serum	Relative electrophoretic values in % of total protein							Turbidity of plasma in units
	Pre-albumin component	Albumin	Globulin					
			alpha-1	*alpha-2*	*beta-1*	*beta-2*	*gamma*	
Fasting	—	54.8	6.7	8.3	13.2	3.5	13.6	3.0
Hyperlipemic†	—	51.1	7.9	9.4	14.0	3.3	14.2	11.3
Post heparin	5.6	53.5	9.0	9.9	8.6	4.3	14.3	7.1

* The values for the pre-albumin component are those obtained after 30 minutes, because at the end of the experiment (55 minutes) this component had migrated beyond the visual field.

† Average values of only five cases because the serum was too turbid in the other three to permit calculation.

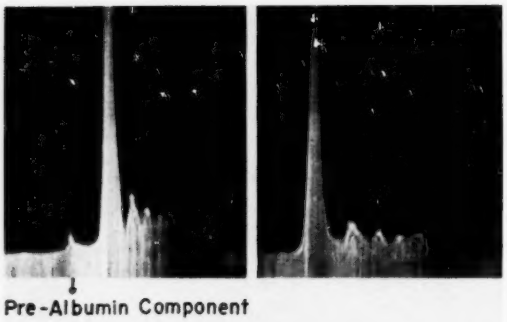

FIG. 3. TWO ELECTROPHORETIC PATTERNS OF AN ALIMENTARY HYPERLIPEMIC SERUM AFTER INTRAVENOUS ADMINISTRATION OF HEPARIN

The photograph on the left was taken 30 minutes after the beginning of the experiment and shows the pre-albumin component. The photograph on the right was taken after 55 minutes when a pre-albumin component was no longer visible.

lipids among the protein fractions nor was any pre-albumin component observed, and the patterns obtained by moving boundary electrophoresis showed no significant changes of the protein fraction values.[2]

DISCUSSION

The most important result of the experiments was the detection of a pre-albumin component, not previously observed in alimentary hyperlipemia after the intravenous injection of heparin. Lever, Smith, and Hurley (8) had found a pre-albumin component, after the injection of heparin, in the fasting plasma of patients with idiopathic hyperlipemia. This pre-albumin component had a slower mobility than the one observed in this study, and it could be easily demonstrated after the normal duration of an electrophoretic experiment by the moving boundary electrophoresis.

In the present study the combined use of paper electrophoresis and moving boundary electrophoresis proved very satisfactory. In the Tiselius electrophoresis apparatus the presence of the pre-albumin peak might have been easily overlooked because of its fast migration, but it could be easily demonstrated on paper strips. Furthermore, the patterns on the paper strips represented specific color reactions, which gave also some information on the chemical nature of the separated fractions. However, from the fact that the pre-albumin component did not appear on the paper strips stained for proteins, one cannot conclude that protein was absent. The method is not sensitive enough to show very small quantities of protein.

That the pre-albumin component is not due to heparin itself is proved by the fact that the injection of 50 mg. of heparin during a fasting state never produced a component migrating ahead of the albumin. Hoch and Chanutin (9) and Blasius and Seitz (10) found that heparin itself formed a peak ahead of the albumin if large quantities of heparin were added to plasma *in vitro*. The concentration of heparin in the present experiments, however, was far too low to produce such a peak. Since the pre-albumin component was well stained with Sudan Black B, a dye specific for lipids, it is established that it contains a relatively large amount of lipids.

To explain the fast mobility of the pre-albumin component and the increase in the mobility of the beta-lipoproteins to that of the alpha globulins, one could assume that a combination of heparin with the lipoproteins occurred (8). Since heparin has a high negative electric charge it could cause an increase in the mobility of the complex toward the anode in the electric field. In such a case the question arises as to whether the 50 mg. of heparin which were injected, if combined with the total amount of circulating lipoproteins, would be enough to cause this phenomenon. Shore, Nichols, and Freeman (11) have shown that human plasma obtained after the intravenous administration of heparin has a lipolytic action, releasing fatty acids from lipoprotein molecules *in vitro*. Since by lipolysis smaller lipoprotein molecules are formed (12) this could contribute to the greater mobility of both lipoprotein classes. However, we did not see in our paper electrophoresis studies any significant increase in alpha-lipoproteins after action of heparin, as might be expected from the studies of Boyle, Bragdon, and Brown (13), indicating an enzymatic conversion of one lipoprotein class to another. Recently, Gordon, Boyle, Brown, Cherkes, and Anfinsen (14) stressed the role of

[2] This observation is at variance with a study reported from this laboratory (8) in which two of seven normal individuals had shown changes in their electrophoretic pattern following injection of heparin. It is possible that these two individuals had not been in a strictly fasting state.

serum albumin in the lipemia clearing reaction, namely the binding of fatty acids released during this reaction. Though fatty acids increase the mobility of albumin (15), the fast moving pre-albumin component is not due to a combination of albumin with fatty acids. In our experiments with paper electrophoresis, the pre-albumin component could be stained with lipid dyes, but not with protein dyes. In control experiments, however, fatty acids increased the speed of migration of pure albumin to a marked extent, but the fatty acid-albumin complex could only be stained by protein dyes and not by lipid dyes.

The electrophoretic changes, although effected by the injection of heparin, are probably not specific for heparin. Oncley and Gurd (16) observed an increase in the rate of electrophoretic migration in beta-lipoprotein preparations which had been stored for a long time. We found that storage of both fasting and hyperlipemic sera in a refrigerator at 5° C. for three to four weeks gave paper electrophoretic patterns similar to those after an injection of heparin. On the paper strips stained for lipids a pre-albumin component was seen, and the beta-globulin lipid material migrated with the speed of the alpha globulins.

SUMMARY

1. The effects of intravenously administered heparin on the plasma proteins and lipids in alimentary hyperlipemia were studied in eight normal individuals by paper electrophoresis and moving boundary electrophoresis.

2. After the injection of heparin analysis of the serum by paper electrophoresis with subsequent staining for proteins and lipids revealed that the lipid band, normally present in the beta globulin area, was then in the area of alpha-2 or alpha-1 globulin. Instead of the lipid band, normally present in the alpha-1 globulin area, lipid material migrated ahead of the albumin.

3. Moving boundary electrophoresis showed a decrease in the beta-1 globulins and an increase in the alpha-2 or alpha-1 globulins or both. A pre-albumin component was also observed.

ACKNOWLEDGMENT

The authors would like to express their appreciation to Dr. Walter F. Lever for advice and suggestions.

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PROCEEDINGS OF THE FORTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 4, 1954

Presidential Address

By CARL V. MOORE

The size of this audience, year after year, is an impressive testimonial to the vital and still growing interest in clinical investigation. To a large extent it exemplifies the great desire of a constantly increasing number of young men to make significant medical contributions of their own, to have full-time careers in academic medicine. As long as this trend continues, one can look to the future with confidence and keen anticipation that our understanding and control of disease will continue to become more complete. The young clinical investigator, however, is confronted with ever increasing competition for the most desirable academic positions, with complex duties that often provide him too little time for creative thought, and with the temptation to seek more money for his laboratory than he can wisely use. These are factors which may eventually dampen enthusiasm or otherwise adversely influence clinical investigation so as to reverse the trend.

I

It has been traditional for young men to start out with the conviction that the only academic jobs really worth having are those as chief of the service or as chairman of the department. We have surrounded those positions with an aura, a sense of reverence which has at least the faint aroma of hero worship. I have heard house officers and research fellows discuss repeatedly the fact that these coveted jobs have increased very little in number while the men who seek them have grown to the size of a small army. They feel that our frontiers are gone, our era of expansion over so that here, as in many European countries excellent men will have to turn away from universities in increasing numbers because opportunities have not kept pace with interest. I would like to argue that the reverse is true, that opportunities have multiplied. To accept and believe this statement, however, we need to keep values straight and not worry about titles.

As faculties have been expanded during the past ten or fifteen years, jobs have been created that are really better than the one the Chairman has. He sets policy, to be sure, is regarded with more esteem by the community, and receives the largest salary; but with the expansion he has had more and more administrative detail, more committee meetings to suffer through, more responsibility to make certain that his associates have what they need, and less free time for his own scientific interests. By contrast, the other one or two professors in his department, the associate and the assistant professors, have more freedom, more time to work on the wards or in the laboratory, more leisure to think. The wise policy of establishing divisions within a department, and of giving division heads a large measure of autonomy, provides the sense of independence that most people need to have. It is difficult to understand, then, why these jobs shouldn't be so satisfactory that the men who hold them would be content with them for life, covet them as the remarkable opportunities they are. Most of them are now coming to offer tenure so that security is not involved. The assistant professor in a medical school often has an income which equals that of a department chairman in other branches of the University. In terms of academic standards, therefore, income is usually good although it obviously won't allow standards of living comparable to those of men in practice. Perhaps the desire to run things, to be "top dog" is so strong in the human race that the security, the opportunity and freedom to work within a University on problems of one's own choosing just won't provide to most people the measure of satisfaction they want from life. I am naive enough, however, to believe that if we would stop blowing up the professor as if he were some superhuman colossus, and would wave a flag more vigorously for the other jobs within a department, we might be able to focus on them as the prizes they are.

II

But if non-administrative departmental positions are to be kept so attractive that men will accept them as lifetime opportunities, both the departmental chairman and his men have a job to do. The former, confronted with increased teaching and service responsibilities himself, must not ask his associates to take over so many of these duties that he destroys their creative effectiveness. His task here is not an easy one. During the period of expansion most of us have recently been through, departments have been asked to take over the responsibility for more patients in their own university hospitals, in veteran institutions and in community hospitals which wanted to affiliate with the University. Any decision by a medical school that the saturation point has been reached is likely to prove unpopular. Most schools have yielded to pressure by increasing the size of their classes, often to the point of greatly reducing faculty-student ratios. The departmental chairman has to be most ingenious under these circumstances in order not to overload his men. In addition, he must give them all the autonomy possible, must make every effort to see that they get the recognition they deserve, and must fight to keep their incomes adequate.

But those of us who have never been chairman of a department often blame the chief of the service too much, and ourselves too little, when free time vanishes. The traps we fall into are fairly obvious, but most men never

learn to avoid them: too many speaking engagements, too many conferences, too many committee meetings. One might follow that by saying: too much talk, too little work. It is reminiscent of the New Yorker story about a maid who quit her job because there was "too much switchin' of the dishes for the fewness of the food." Most men appreciate the recognition provided by these outside assignments, of course, and have a responsibility to accept their share, but the proper balance is hard to keep. Then there is the temptation to take oneself out of the academic income bracket by seeing more and more private patients or by assuming additional extraneous duties mostly because of the money involved. The price we have to pay in medicine, particularly in clinical departments, for the great privileges of an academic life, for the stimulating association with brilliant colleagues, for the opportunity to do just what we want to do, is so much less than that required of our other university associates that most salary protests have a hollow ring. Income bought at the expense of free time for creative work will certainly, for the investigator, seem like a poor investment when the age of 65 or 68 rolls around.

III

The danger also exists that each division head, when given a large measure of autonomy, will be tempted to build a little empire of his own without regard for the structure of the department as a whole. There is a disease which is fostered by this system. As departments increased in size, most medical schools had to use for salaries the money that formerly had supported research. Governmental and foundation research funds, fortunately, became more plentiful and filled the hole. Each man was told to apply for his own research grants. There then developed what may be called the "centrifuge-scintillation counter-Beckman disease." Formerly, one or two centrifuges would suffice for a whole department. Now, every laboratory must have at least one. This disease has characteristic symptoms and signs. There is acute depression if the laboratory budget is less than $100,000 a year. There is unhappiness if there are so few fellows and technicians that the principal investigator has to do any work himself. No experiment involving radioactive isotopes can possibly be undertaken unless the laboratory has at least one counter from every instrument company in the field. There are more pieces of equipment than ideas. There is an amazing lack of any sense of responsibility for the wisest possible use of the funds. I think this preoccupation with equipment and large operations unfortunate, not only because of the waste involved, but because it tends to generate a false conception of research. It involves young men in the task of using instruments to pick up little pieces of information that have been passed over by more original investigators, encourages them to remain in laboratories past the time when they should have realized that their talents lay principally in other directions, or denies them the chance to explore more exciting leads because a longer time might be needed to obtain publishable results.

IV

This harangue was prompted only by: 1) the hope of convincing young men that the medical academic ladder is not as clogged as is the membership of this Society, 2) the desire to plea that non-administrative departmental jobs be organized to preserve the freedom necessary for active investigative programs, and 3) by the fear that unwise use of research funds may discourage the proverbial goose. Lastly, I would like to talk briefly about the meeting place of this Society. The meetings of the American Society for Clinical Investigation exert a tremendous influence on young men, and on clinical investigation as a whole in the United States. Ninety-two men or 34 per cent of our membership now come from the middle west or far west (Figure 1). House officers and fellows from these areas, year after year make the pilgrimage to Atlantic City at great expense to themselves or to their department, but only a fraction of them can make the trip. The time has come, it seems to me, for us to recognize the "American" in our name, to meet in other sections of the country as well as here. On purely democratic grounds there is no right at present to ask that more than one out of every three meetings be held in a central location. That, at least, should be done so that the splendid influence of these sessions can be spread over a broader section of the country. Men have discussed such a plan on the Boardwalk for years; councils have debated it; the time is ripe for one of your presidents to recommend it from here.

FIGURE 1

ABSTRACTS

Observations on the Capacity of Serum Alpha Globulin to Bind Thyroxin in Vitro. EDWIN C. ALBRIGHT, FRANK G. LARSON and WILLIAM P. DEISS, Madison, Wis. (Introduced by Ovid O. Meyer).

Previous studies demonstrated the association of thyroxin with a specific serum alpha globulin *in vivo*. This specific alpha globulin binding was also demonstrated *in vitro* using I^{131} labelled thyroxin.

The thyroxin binding capacity of this protein was studied in hyperthyroid, euthyroid and hypothyroid subjects. Quantities of I^{131} labelled thyroxin were added to serum to yield concentrations of from .05 to 1.0 micrograms/milliliter. The thyroxin serum mixtures were subjected to paper zone electrophoresis at pH 8.6 (0.1 M veronal buffer). Following electrophoresis the distribution of radioactivity was determined.

In all sera the thyroxin appeared in the alpha globulin zone. At the lowest concentrations used (.05 to .1 micrograms/milliliter) only a very small amount of thyroxin appeared in the albumin. As the concentration of thyroxin was increased from .1 to 1.0 micrograms/milliliter a greater proportion of thyroxin appeared in the albumin as the alpha globulin binding capacity appeared to become relatively saturated. The concentration of thyroxin required to produce equal distribution of radioactivity between the alpha globulin and albumin zones has been used as a convenient measure of alpha globulin thyroxin binding capacity. This equilibration was found to occur with concentrations of .2 to .5 micrograms/milliliter in the hyperthyroid sera, from .6 to .8 micrograms/milliliter in the euthyroid and approximately 1 microgram/milliliter in the hypothyroid. Addition of iodide *in vitro* in concentrations from .001 to 10 micrograms/milliliter did not affect the thyroxin binding capacity. Administration of Lugol's solution to the hyperthyroid subjects appeared to increase thyroxin binding capacity somewhat.

It appears that *in vitro* thyroxin binding capacity of serum alpha globulin varies inversely with the state of thyroid gland activity. This is assumed to indicate that this protein in hyperthyroid subjects is relatively saturated with endogenous thyroxin, and conversely relatively unsaturated in the hypothyroid.

Water Content of Hyperlipemic Serum. MARGARET J. ALBRINK, PAULINE M. HALD and EVELYN B. MAN, New Haven, Conn. (Introduced by C. N. H. Long).

The displacement of serum water by the lipids of lactescent serum was forcefully demonstrated in a patient whose low serum electrolyte values were directly attributable to the large volume occupied by the insoluble lipids of his serum. The patient, an 18 year old boy with long-standing skin xanthomas, was admitted with mild diabetic acidosis and markedly lactescent serum. His serum sodium was 103 mEq./L. and his serum chloride was 75 mEq./L., values quite out of keeping with the mildness of his illness. When the insoluble fat was removed from the serum by ultracentrifugation, the sodium content of the clear subnatant fluid was 135 mEq./L. and the chloride 100 mEq./L. The serum water determined gravimetrically was 73 gm./100 ml. serum. The total fatty acids were 526 mEq./L., and the cholesterol 1219 mg. per cent.

An ensuing study of sodium, water, and lipid content of several lactescent sera revealed that as lipids increased there was a growing discrepancy between serum sodium and sodium recalculated in terms of serum water.

These findings prompted a search for a rapid and simple method for the estimation of serum water. A preliminary study indicates that a method involving the measurement of freezing-point depression of serum before and after addition of a known amount of dry sodium chloride meets this requirement. The determinations, requiring 2 ml. of serum, were accomplished by means of a freezing-point osmometer calibrated to give the osmolarity of the fluid analyzed.

Determinations were carried out on 10 different samples of biologic fluid, including several lactescent sera. The water content of these samples ranged from 78 per cent to 99 per cent. The values obtained osmotically agreed within one per cent with those obtained by the gravimetric method.

This method has the advantage of affording a direct estimation of serum osmolarity.

Elevated SPCA (Convertin) Complex in Pregnancy. Its Possible Role in Pathogenesis of Thromboembolism. BENJAMIN ALEXANDER,* LILLIAN MEYERS, ROBERT GOLDSTEIN, VICTOR GUREWICH and LESTER GRINSPOON, Boston, Mass.

Although abnormal coagulation has been incriminated as a basic cause of thromboembolism, convincing evidence is lacking. Recent identification of new clotting factors raised the possibility that abnormalities in one or more of these substances might underly the development of thromboembolic disease. To explore this, the pregnant state was selected because it constitutes a common, temporary, reversible, relatively physiologic "deviation" which is frequently complicated by thromboembolism.

In a study of 57 pregnant women the plasma Spca complex was distinctly elevated, prothrombin less frequently and to a lesser extent. The Spca tended to increase further as term approached, and reverted toward normal within days after delivery. The average Spca of the gravid was 189% of normal (Owren method) compared with 82% for the non-pregnant group (41). In ten patients the Spca values were three to five fold the

control average; in five additional patients the levels exceeded the non-pregnant average by more than five fold. In 24 subjects the average level three to five days postpartum was 114% contrasted with 150% shortly before delivery. In two individuals, striking Spca elevations persisted longer.

Prothrombin was less significantly altered. However, in nine women the prothrombin was between 140–180% of normal compared with an average level of 98% (range 58–128) in the controls.

The data support the concept of hypercoagulable blood in pregnancy. The role of Spca in accelerating thrombin evolution, its activation by thromboplastin, and its effectiveness in inducing intravascular thrombosis in animals (Wessler) are known. Excesses of this clotting factor, together with venous stasis and increased platelet adhesiveness already demonstrated in pregnancy (H. P. Wright), provide a delicately balanced stage which could readily be triggered to induce thrombosis by liberation into the circulation of thromboplastic material from the gravid uterus. The latter is known to occur.

Chronic Hypercapnia: A Specific Respiratory Depressant in Chronic Pulmonary Disease and Other Conditions. JAMES K. ALEXANDER, JOHN A. WOOD and JOHN R. WEST, New York, N. Y. (Introduced by Dickinson W. Richards, Jr.).

That patients with pulmonary emphysema and chronic respiratory acidosis have a diminished ventilatory response to carbon dioxide inhalation seems well established. Possible mechanisms are (1) increased buffering capacity of the blood associated with (a) elevated plasma bicarbonate or (b) polycythemia; (2) failure of the chest bellows to respond normally to the central nervous stimuli; (3) diminution in the sensitivity of the nervous regulatory mechanism to the carbon dioxide-hydrogen ion stimulus as a result of (a) congestive heart failure, (b) chronic acidosis, (c) chronic anoxemia or (d) chronic hypercapnia. Each of these possibilities has been investigated by relating the effective alveolar ventilation to the changes in arterial carbon dioxide tension and hydrogen ion concentration produced by addition of carbon dioxide to the inspired air.

Since the ventilatory response in these patients was reduced relative to the stimulus in terms of the hydrogen ion concentration as well as the carbon dioxide tension, increased buffering capacity of the blood did not account for the results. The diminished ventilatory response was independent of congestive heart failure and mechanical ventilatory impairment. Further observations on patients suffering from chronic anoxemia secondary to cyanotic congenital heart disease, or chronic metabolic acidosis associated with uremia, revealed no reduction in sensitivity to the carbon dioxide-hydrogen ion stimulus under these conditions. However, patients with chronic hypercapnia and an increased alkaline reserve associated with metabolic alkalosis were found to have substantial reductions in sensitivity to the carbon dioxide-hydrogen ion stimulus.

It is concluded that chronic hypercapnia per se results in a diminished sensitivity of the respiratory nervous regulatory mechanism to the carbon dioxide-hydrogen ion stimulus.

An Investigation of the Validity of "Insulin-resistance" in Diabetes Mellitus. LOUIS K. ALPERT, ALVIN E. PARRISH and KARL P. KOLB, Washington, D. C. (Introduced by Theodore J. Abernethy).

This study was undertaken to determine whether inhibition of insulin activity could be demonstrated in diabetic subjects.

A constant state of carbohydrate utilization was established by a continuous intravenous infusion of 240 mgm. of glucose per minute. When the blood glucose levels had stabilized, regular insulin, 5 units per M^2, was injected intravenously. The rate of fall in blood glucose (mgm. per 100 cc. per minute) during the subsequent 45 minutes of continued infusion was used as the index of response to insulin.

49 diabetic and 31 non-diabetic patients were studied. In the non-diabetics, the mean index was 1.06 (S. D. $\pm$ 0.36), and in the diabetics was 1.51 (S. D. $\pm$ 1.28). The individual indices in the non-diabetics ranged from 0 to 2.0, with 85% falling between 0.5 and 1.5. In the diabetics the range was 0 to 6.5, with 48% falling between 0.5 and 1.5, and 35% between 1.5 and 2.5. The values of the indices were unrelated to the blood sugar levels of the patients, or to the daily requirements for insulin.

It is concluded that these diabetic patients, with the method employed, appeared to show an equal or greater response to insulin than did the non-diabetic individuals. These observations, if valid, would be compatible with the concept that a) diabetes mellitus may be due essentially to an endogenous lack of insulin production, and b) variations in insulin requirements may be associated with independent gluconeogenetic factors.

Exchange of Cations, Respiratory Gases and Carbohydrate by Intact Human Muscle. R. ANDRES, K. L. ZIERLER,* G. CADER, A. S. GHRAYYIB and J. L. LILIENTHAL, JR.,* Baltimore, Md.

As a first step in examining metabolic events in skeletal muscle in man, serial measurements in six subjects of respiratory gas exchange and movement of certain carbohydrates and cations have been made in the human forearm. Net exchange of metabolites was determined as the product of arterio-venous concentration difference and blood flow. The latter was determined by constant injection of T-1824 into the brachial artery and continuous sampling of venous outflow from a catheter placed deep in forearm muscles. This method demonstrated reasonably constant flow under "resting" laboratory conditions and a brisk response to exercise of forearm musculature.

In some subjects oxygen consumption and carbon dioxide production and their exchange ratios were relatively steady. In others random minute-to-minute variations were of extraordinary magnitude and were out of

phase. For the entire group, oxygen uptake per forearm ranged from 0.9 to 5.1 ml./min., and carbon dioxide production from 0.3 to 6.3 ml./min., with exchange ratios varying from 0.12 to 2.05.

Traditionally, *in vitro* studies have indicated that the major part of oxygen utilized by resting muscle is consumed in oxidation of glucose. Anaerobic degradation of glucose is accounted for largely by the appearance of lactate. However, in these studies such a balance was demonstrated in only a few instances. In the majority of cases none or little of the oxygen used could be accounted for by aerobic glucose utilization.

Potassium appeared to leave the forearm in small quantities during "rest", and the rate of escape was accelerated by exercise. Net exchanges of sodium were random in direction.

These studies, designed to provide basic data for subsequent assessment of altered muscle metabolism, indicate remarkable minute-to-minute instability of metabolic exchanges in "resting" muscle and suggest that carbohydrate metabolism does not always account for the major fraction of oxygen consumed.

A Comparison of Dextran and PVP in Human Volunteers. JOHN B. ARMSTRONG, Winnipeg, Canada. (Introduced by J. Doupe).

Five hundred or 1000 ml. quantities of 6% British or Swedish dextran were administered to 12 normal subjects, and of 3.5% polyvinylpyrrolidone to 12. Serum and urine determinations, together with haematocrit and plasma protein concentrations were carried out before, immediately after, and at 24 hour intervals following infusion.

The serum concentration fell more sharply with Swedish than with British dextran. PVP showed a more rapid early decline in serum concentration but was better maintained after 24 hours. These changes were reflected in the urinary excretion. Both dextran and PVP caused a fall in haematocrit and plasma protein concentration immediately following the infusion, which disappeared as their blood levels fell.

Seven dextran subjects received a prior venesection, and showed a haematocrit dilution persisting well beyond that found with dextran alone. To elucidate, 10 subjects underwent venesection only. They showed a delayed dilution of the haematocrit suggesting that the persistent dilution was due to the early effects of dextran and the later effects of venesection. The plasma protein dilution following venesection and dextran was not different from dextran alone. This may be explained by the observed rapid disappearance of plasma protein dilution following venesection only.

Seven subjects received a venesection followed by 500 ml. isotonic saline. The haematocrit and plasma protein dilution was not significantly different from venesection only. This indicates a rapid loss of saline from the circulation in contrast to the slower loss of dextran and PVP.

The discordant shifts of haematocrit and plasma protein dilution suggest that neither is a reliable index of plasma volume changes following haemorrhage.

Adrenal Cortical Activity in Normal Men on Diets of Low Sodium Content. B. J. AXELRAD and J. A. LUETSCHER, JR.,* San Francisco, Calif.

When dietary sodium intake of normal men was reduced from 150 to 15 mEq. per day, urine sodium fell abruptly to low levels by the third day and reached a minimum by the fifth day of the low sodium diet. Insignificant changes in daily creatinine excretion and in inulin clearance indicated that tubular reabsorption of sodium increased during this period of low sodium intake.

Previous work on edematous patients showed that chloroform extracts of urine acidified to pH 1.0 contained high sodium-retaining activity demonstrable by bioassay. Sodium-retaining activity could be demonstrated in normal men on unrestricted sodium intake only if the urine was subjected to prolonged acid hydrolysis, and if large dosages of extract were assayed. Measured under these conditions, the normal control output of activity was increased five fold by the third day on low sodium diet, and remained at this level until normal sodium intake was resumed on the 7th day.

The material responsible for the sodium-retaining activity had the chromatographic mobility characteristic of the sodium-retaining corticoid isolated from the urine of edematous patients with heart failure or nephrosis and of "electrocortin" from adrenal cortical extract.

The increased output of sodium-retaining hormone was not accompanied by any significant change in output of 17-ketosteroids or 17-hydroxycorticoids measured by Forsham and Island. Diets of low sodium content appear to stimulate primarily the sodium retaining hormone produced by the adrenal cortex.

The Use of Monocalcium Disodium Ethylene Diamine Tetra-acetic Acetate (CAEDTA) in the Treatment of Adult Lead Intoxication. WILLIAM H. BAKER, Boston, Mass. (Introduced by Joseph C. Aub).

Recently a new chelating agent, monocalcium disodium ethylene diamine tetra-acetate (CAEDTA), has been introduced for clinical trial in heavy metal poisoning.

CAEDTA has a strong affinity for lead and exchanges its calcium for lead to form a PbEDTA complex which is then excreted unchanged via the urine. We have administered this compound intravenously in a dosage of 0.5 gm. per 30 pounds of body weight to six patients with chronic lead poisoning and to three normal control patients. All six patients with chronic plumbism prior to treatment showed increased urinary lead and coproporphyrin levels. All patients had anemia with stippling of the red cells and abnormal fragility tests. CAEDTA was administered to each patient on consecutive days for periods of four to eighteen days. On the first day of administration there was a marked increase in urinary lead excretion and a simultaneous lowering of coproporphyrins. On succeeding administrations there was a gradual

decrease in the amount of lead excreted while urinary coproporphyrins remained at normal levels. During the period of therapy all patients exhibited a rise in hemoglobin, stippling disappeared, and fragility tests returned to normal. In the three normal control subjects CAEDTA was administered on only one day with an increase in urinary lead on that day and no change in the coproporphyrin. Metabolic balance studies of electrolytes were performed on two patients with chronic lead poisoning. No alterations in metabolic balance occurred during administration of this compound either in a six or eighteen day study period.

These studies indicate that CAEDTA is an effective way of mobilizing deposited lead with simultaneous improvement in the patient's condition and no evidence of toxicity.

Urinary Concentrating Process during Antidiuresis in Renal Disease. DAVID S. BALDWIN, HAROLD J. BERMAN, and HENRY O. HEINEMANN, New York, N. Y. (Introduced by Herbert Chasis).

Fixation of urinary specific gravity in renal disease is generally assigned to loss of renal tubular concentrating ability. In this study the urinary concentrating process has been examined by the cryoscopic method, and a tubular mechanism for the production of a hypertonic urine has been shown to persist in the course of various renal diseases.

Observations of glomerular filtration rate (GFR) and osmolarity of plasma and urine were made in the hydropenic state in 20 patients during Pitressin administration and with osmotic diuresis induced by mannitol. The quantity of water abstracted from the isosmotic glomerular filtrate in the formation of osmotically concentrated urine was calculated as the difference between the osmolar clearance and the urine flow and designed as T_{H_2O}.

In each case a relatively constant $T^c_{H_2O}$ was attained, ranging in these patients from 0.8 to 8.2 ml. per min., while the range of GFR was from 12 to 96 ml. per min. In general, there were proportionate reductions in $T^c_{H_2O}$ and GFR with an average $T^c_{H_2O}$/GFR ratio of 0.056 as compared to the normal ratio of 0.051 ± 0.015. However, in 2 patients recovering from acute renal failure there was a disproportionate impairment of $T^c_{H_2O}$ as manifested by $T^c_{H_2O}$/GFR ratios of 0.017 and 0.008, while in 2 acute nephritics the ratios were 0.086 and 0.072.

Lack of correlation between urinary osmolar concentration and urine specific gravity as determined by the urinometer was demonstrated repeatedly. In no case did urine osmolarity fail to exceed plasma osmolarity, even in the presence of "fixed" specific gravity.

It is concluded that a maximal tubular reabsorptive rate for free water in the formation of a hypertonic urine is demonstrable in renal disease. With few exceptions this process deteriorates in proportion to the diminution of glomerular filtration, suggesting loss of functioning units rather than specific tubular dysfunction.

Radioactive Iodine for Chronic Lung Disease. BERNARD A. BERCU and HARVEY N. MANDELL, St. Louis, Mo. (Introduced by Robert J. Glaser).

The present treatment for chronic pulmonary insufficiency is usually disappointing and frequently of only temporary benefit. Since one fundamental defect in chronic lung disease is a deficiency in the supply of oxygen to the body, it was thought worthwhile to determine the effect of lowering the basal requirements of the tissues for oxygen by making patients with chronic lung disease relatively hypothyroid.

Ten patients with pulmonary emphysema and/or fibrosis were selected. These patients had been treated repeatedly by the usual methods and were still markedly incapacitated either at rest or upon slight exertion. Since antithyroid treatment could not be expected to alter the fundamental pulmonary lesion, objective changes in lung function were not anticipated. In evaluating the effect of therapy, reliance was therefore placed upon standard exercise tolerance tests and upon subjective changes. Patients were considered significantly improved if they were able to perform activities which previously were intolerable because of dyspnea. Those who did not give evidence of increased exercise tolerance, even though they were improved subjectively, were considered as only questionably improved.

Of the ten patients treated, three were initially made hypothyroid with Tapazole. The other seven were given I^{131} on one or more occasions. All patients were euthyroid prior to therapy. Seven patients were found to be significantly improved, one was questionably benefitted, and two were unchanged. In two patients severe dyspnea developed after the Tapazole was discontinued in preparation for the use of I^{131}. This dyspnea again disappeared in one patient after establishment of hypothyroidism. The other patient expired during the interval.

Influence of Age and Size of Bacterial Population on the Rapid Bactericidal Action of Penicillin. CARL BERNTSEN, New York, N. Y. (Introduced by Ralph Tompsett).

Relapse of infections caused by penicillin susceptible bacteria following penicillin therapy is an apparent inconsistency with its rapid bactericidal action on these same bacteria *in vitro*. The commonest explanation of this seeming paradox has been based on the intervention of an inflammatory lesion, with presumed failure of its penetration by metabolites or drug. These hypotheses have little experimental support.

In preliminary studies it was found that the total population of bacteria in lesions of experimental animals and man were higher than those customarily tested *in vitro*. Moreover, it appeared that the ages of individual members in such populations varied considerably. Accordingly, an investigation was made of the influence of age and size of bacterial populations on the bactericidal action of penicillin.

The test microorganism employed was a highly penicillin susceptible strain of Group A Streptococcus hemo-

Plasma levels of 17-hydroxycorticosteroids were normal in the patients with liver disease. After a standard infusion of hydrocortisone in twelve patients with liver disease and in eleven normal subjects, the 17-hydroxycorticosteroids disappeared from the plasma in a logarithmic fashion, at a rate which was proportional to the functional capacity of the liver as measured by the degree of BSP retention. In patients with hepatitis, serial studies demonstrated an increased rate of 17-hydroxycorticosteroid removal as the liver function improved.

As compared with hydrocortisone, tetrahydrocortisone, which has been shown to be the major 17-hydroxycorticosteroid excreted in the urine, disappeared from the plasma at a much more rapid rate which was independent of liver function.

The rise in the plasma 17-hydroxycorticosteroid levels in response to ACTH stimulation in the patients with liver disease was within normal limits.

The daily urinary excretion of 17-hydroxycorticosteroids of the patients with liver disease was 3.5 ± 0.4 mg. as compared with 5.5 ± 1.2 mg. for the normal group. These results differ from those of other workers who used less specific methods for the measurement of adrenalcortical steroids.

These data indicate that the removal of 17-hydroxycorticosteroids from the plasma is dependent upon the functional state of the liver. Hence the plasma level of these steroids depends at least in part on the functional state of the liver as well as the rate of adrenalcortical secretion.

The Determination of Pressure Gradients in the Vascular System of Man. ROBERT A. BRUCE,* CURT A. WIEDERHIELM, JAMES J. PAMPUSH and HENRY J. KOWALSKI, Seattle, Wash.

Recording of instantaneous pressure gradients between any two points in the vascular system previously used in animal studies has been simplified and applied to humans during cardiac catheterization.

Direct pressures from two sites, the instantaneous difference between these sites and the electrocardiogram can be simultaneously recorded on a Sanborn Poly-viso. A double lumen catheter, whose orifices are 10 cm. apart, is used with Statham strain gages. Instantaneous pressure differences are derived by electrical subtraction of one impulse from the other and inscribed directly as a gradient in mm. Hg. Electrical calibration can be done repeatedly. Gradients of 0.5 mm. Hg are readily determined. Limiting factors appear to be the physical properties of the catheters insofar as they contribute to resonance and damping by their distensibility characteristics.

The pressure gradient between inferior vena cava and right auricle may reverse during each heart cycle. Flow here may be discontinuous or phasic.

Pressure gradients across tricuspid and pulmonic valves and through intracardiac defects can be obtained. Premature ventricular systoles markedly alter the gradient between right ventricle and right auricle and indicate incomplete closure of the valve during the abnormal contraction.

Respiratory variations induced by breathing, coughing, and the Muller and Valsalva maneuvers affect pulmonary arterial and "capillary" pressures, but have little effect on the gradient within the pulmonary artery. Since arterial-"capillary" gradients may be altered by changes in intrathoracic pressure, pulsatile flow through the capillary bed is modified by respiration. The effects of mitral valve disease on PA–PC pressure gradients can be observed.

It is concluded from these preliminary observations that simultaneous differential pressure measurements during cardiac catheterization are simple, reliable and valuable adjuncts to the studies usually made. The technique is applicable to the instantaneous differentiation of any phenomena that can be recorded electrically with this equipment.

Synergistic Effect of Polymyxin B and Oxytetracycline or Tetracycline Upon Growth of Pseudomonas Aeruginosa. PAUL BUNN,* LEONARD CANARILE and WINIFRED OSBORNE, Syracuse, N. Y.

Forty-three strains of *Pseudomonas aeruginosa* derived from human infections were tested for sensitivity to eight antimicrobial agents. Maximal inhibiting concentrations were determined by the serial dilution technic, using a 10^{-5} dilution of organism as the inoculum. All strains were resistant to more than 6.0 micrograms per ml. of penicillin, streptomycin, chlortetracycline, tetracycline, oxytetracycline, chloramphenicol and bacitracin but were sensitive to less than 3.5 micrograms polymyxin B per ml. The maximal inhibiting concentration of polymyxin B failed to control growth of all strains after four hours when an undiluted culture was used as the inoculum.

Complete inhibition of growth of the undiluted culture of pseudomonas was achieved for more than eight hours when either one-quarter the sensitivity level of polymyxin was combined with the maximal inhibiting concentration of oxytetracycline or tetracycline, or when one-half of those concentrations was employed. This effect was uniformly greater than that observed with double the maximal inhibiting levels of the three agents singly, both in duration of effect and in number of bacteria killed.

This synergistic effect upon inhibition of growth of *Pseudomonas aeruginosa* could not be demonstrated with four other combinations of antimicrobial agents.

Sterilization of the urine of four humans with chronic *Pseudomonas aeruginosa* urinary trace infections was accomplished with a dosage regimen of 25 milligrams polymyxin B once daily parenterally combined with 2 grams orally of either tetracycline or oxytetracycline. In a fifth case a few colonies of pseudomonas persisted in the urine after seven days but their ability to produce green pigment was lost. Toxicity to this regimen was not observed and no strain of *Pseudomonas aeruginosa* became resistant to the three agents during the period of study.

The Tissue Utilization of Endogenous Thyroid Hormone in Human Subjects. BELTON A. BURROWS, THEODORE PETERS and JOSEPH F. ROSS,* Boston, Mass.

We have estimated the peripheral utilization of endogenously produced thyroid hormone in human subjects from the amounts of iodide being released from the catabolized hormone. The values depend on the size of the extrathyroidal iodide pool, and the rate at which it is being turned over.

Such studies are possible if added iodide equilibrates rapidly with the iodide pool in comparison to the turnover rate. In five myxedematous patients, in whom equilibration might be slow, the radioiodide spaces one hour after an intravenous tracer dose of I-131 averaged 1.5 times the simultaneously determined radiosulfate spaces, taken as an index of extracellular fluid volume, and approached maximum values. This indicates rapid equilibration of iodide returning from the tissues with the iodide pool.

The turnover rate of the iodide pool was indicated by the rate of decrease of its specific activity (I-131/I-127), due to the release of I-127 from catabolized thyroid hormone in the tissues. It averaged 22.4 percent per hour in the normal subjects as compared with 37.9 percent per hour in the hyperthyroid individuals. The iodide pool was virtually constant in individual patients during the period of study, averaging 48 micrograms of I-127 in the normal group. The average value for the hyperthyroid group was considerably higher, 295 micrograms of I-127, due mainly to an increased serum concentration of inorganic I-127.

The estimated amounts of hormonal iodide released from the tissues in the normal group (8.5 micrograms per hour) would be equivalent to the daily utilization of 300 micrograms of levo-thyroxine. In the hyperthyroid group the average peripheral iodide release was ten times greater (73.4 micrograms per hour), which would be sufficient to account for the observed hypermetabolism of hyperthyroidism.

Ferrokinetics in Anemia of Infection. J. A. BUSH and G. E. CARTWRIGHT,* Salt Lake City, Utah.

Ferrokinetic studies of (Fe^{59}) have been made in five patients with anemia associated with chronic infection and in seven normal subjects. Ten to 20μc of Fe^{59} containing less than 6 μg of total iron were incubated with the individual's own plasma, and the labelled plasma was injected intravenously. Blood volumes were determined by means of I^{131}-labelled human serum albumin.

In none of the patients was clinical jaundice present, and there was no detectable increase in urobilinogen excretion in the stools. The mean volume of packed red cells in these patients was 37 ml./100 ml., the plasma iron 38 μg per cent, and the reticulocytes 1.4 per cent.

In the patients with infection the half-time of plasma Fe^{59} disappearance was 0.50 hours; the plasma iron turnover rate was 0.64 mg./kg./24 hours; the red blood cell iron turnover rate was 0.62 mg./kg./24 hours; the red cell iron renewal rate was 1.9 per cent/24 hours. In the normal subjects the half-time of plasma Fe^{59} disappearance was 1.43 hours; plasma iron turnover rate, 0.52 mg./kg./24 hours; red cell iron turnover rate, 0.48 mg./kg./24 hours; red cell iron renewal rate, 1.2 per cent/24 hours.

Incorporation of radio iron into the erythrocytes occurred somewhat more rapidly in the patients with infection as compared with the normal individuals. The per cent of the administered dose which ultimately appeared in the erythrocytes was the same in each group.

The above data suggest that the erythrocyte survival time of patients with chronic infections is slightly shortened (60 per cent of the normal), and that erythrocyte production although slightly increased above the normal (approximately 1.3 times) is not increased sufficiently to prevent the development of a mild degree of anemia. When consideration is given to the fact that a normal marrow is capable of increasing its production rate 6 to 8 fold, it is apparent that there is a marked inhibition of erythropoiesis in such patients.

A Study of the Electrical Activity of Intact and Partially Mobilized Human Ureters. HARVEY R. BUTCHER, JR. and WILLIAM SLEATOR, JR., St. Louis, Mo. (Introduced by Carl A. Moyer).

Little is known regarding the part played by abnormalities of ureteral activity in the genesis of ills of the upper urogenital tract. Acute hydroureter and hydronephrosis have become increasingly important medical problems as uretero-intestinal anastomosis has become one of the common surgical procedures.

A method of intraureteral electroureterography has been developed in order to investigate the effect of an uretero-intestinal anastomosis upon the activity of the ureter. The method entails bipolar recording of ureteral peristaltic action potentials from intraluminal electrodes. After performing extensive preliminary investigations upon the anesthetized dog, the method was applied to the study of ureteral activity in man before and after surgical manipulation of the ureter.

The investigation of normal human ureteral activity has led to a number of new observations:

(1) The shape of the recorded ureteral peristaltic action potential in man is similar to that of the dog. Control experiments indicate that the voltage-time contour recorded by this method represents actual changes in ureteral smooth muscle membrane potential. There is a "shoulder" during electrical recovery similar to that of action potentials recorded intracellularly from cardiac muscle.

(2) A large slow voltage change accompanies the passage of urine by the electrodes.

(3) Variations in the velocity of peristaltic waves correlate with changes in the shape of the recorded action potentials and with changes in the length of the interperistaltic wave interval.

The disturbance in ureteral function attending uretero-intestinal anastomosis is a failure of propagation of the

peristaltic wave through surgically disturbed segments of the ureter. Maximum duration of this disturbance in conduction is as yet unknown; however, it lasts at least the two to three days the electrode assembly has been left in the ureter postoperatively. Frequency and velocity of propagation of ureteral peristalsis in the undisturbed section of the ureter were unaltered by uretero-intestinal anastomosis.

Effect of Maintenance Cortisone Therapy on the Metabolic Response to Surgery. EVAN CALKINS and DONALD M. MITCHELL, Boston, Mass. (Introduced by Walter Bauer).

Requirements for the normal response to surgery are not entirely understood. An opportunity for study was provided by the successive development of bilateral inguinal herniae in a patient with rheumatoid arthritis maintained on 75–100 mg. oral cortisone. On two occasions, under general and local anesthesia, respectively, he underwent herniorrhaphy while on the metabolic ward. Cortisone dosage was unchanged throughout each experiment. Diet was unchanged except that he was unable to eat on the day of ether anesthesia. The metabolic response was compared with that caused by subsequent sudden increase in cortisone to 250 mg. per day, and with balances which had been obtained on this patient prior to and immediately following initiation of cortisone therapy two years previously.

Data showed that surgery was followed by negligible change in nitrogen, potassium, calcium, and phosphorus balances and in creatine excretion. There was, however, striking sodium retention. A similar metabolic pattern accompanied onset of steroid therapy and sudden increase in dosage. The first operation was followed by a sudden fall in 17-ketosteroid excretion from 11 to 4.6 mg. and in formaldehydogenic steroid excretion from 36 to 16 mg. per day. Similar determinations were performed at the time of the second operation.

These results indicate that this patient (presumably because of his depletion) reacts to the stress of surgery and to increase in cortisone primarily with sodium retention, rather than with an acute catabolic response as seen in normal individuals. The fact that he displayed this characteristic response following surgery, despite suppression of adrenal cortical activation by constant cortisone administration, suggests that the response may, in this instance, reflect altered metabolism or increased "utilization" of steroids.

A Study of the Nature of the Peripheral Resistance in Patients with Hypertension. FRANCIS S. CALIVA, JAY F. HARRIS and RICHARD H. LYONS,* Syracuse, N. Y.

This study was undertaken to determine if the vascular bed of the toe participates in the increased resistance in hypertension and if the nature of this resistance differs in the various stages and types of hypertension.

Patients were of four clinical types. The first group was made up of 18 normal patients; group two, of 11 hypertensive patients without eyeground changes or evidence of renal disease; group three, of 7 patients with hypertension and moderate retinopathy and renal disease; and group four, of 7 patients with marked retinopathy and/or azotemia. In none was there evidence of cardiac failure or peripheral vascular disease. Studies were carried out in a constant temperature room with the patient in the supine position. Peripheral resistances were calculated by dividing the mean blood pressure by the blood flow. Blood flows to the toe were determined plethysmographically before and after posterior tibial block.

It was found that in the resting state the peripheral resistances of each of the hypertensive groups was higher than that of the normotensives. After the abolition of local neurogenic tone, resistances of the hypertensive patients fell into three groups which correlated well with the clinical disease. The mean peripheral resistance in the normotensives after block was 7.5 units. In the hypertensive patients, group two (see above) had an average peripheral resistance of 7.2 units, group three of 12.2 units, and group four of 20.4 units.

It is suggested that the increased peripheral resistance in hypertension includes the vascular bed of the toe. This increased resistance in individuals without renal disease apparently is mainly on a neurogenic basis since it can be reduced to normal levels following a local sympathetic block. In patients with renal disease, other mechanisms must be operative.

Evidence Concerning the Relationship of the Amygdaloid Nuclear Region to the Regulation of the Circulation and Emotion in Man. WILLIAM P. CHAPMAN,* HEINZ R. SCHROEDER, GEORG GEYER, MARY A. B. BRAZIER, CHARLES FAGER, JAMES L. POPPEN, HARRY C. SOLOMON and PAUL I. YAKOVLEV, Boston, Mass.

The effects of electrical stimulation of the amygdaloid nuclear region in the temporal lobe on the continuous recording of blood pressure and heart rate and on feeling states and motor behavior have been determined in four awake epileptic patients in whom this area was afterwards coagulated for therapeutic reasons. The patients were selected by a group of psychiatrists for psychosurgery for the treatment of psychomotor seizures or unmanageable assaultive behavior. A four-needle electrode, implanted in the amygdaloid area under stereotaxic control, made possible observations over several days as well as the localized coagulation procedure.

In one patient, no consistent responses were obtained. In three patients, electrical stimulation with 20–60 cycles per second, 1 millisecond pulse duration and 8–14 volts produced a 20–100% increase in heart rate. In two patients, stimulation with the same electrical characteristics, except for 12–17 volts, caused a 50–83 mm. Hg rise in systolic and 33–60 mm. Hg rise in diastolic blood pressure. The elevations in heart rate and blood pressure could be produced independently of changes in emotional expressions, but were more marked when obtained in association with feelings of anxiety and fear and somatic responses regarded as appropriate for such subjective changes. Bilateral pupillary dilation was a frequent accompaniment of the circulatory responses. The char-

acter of the circulatory effects suggested that some responses were mediated by direct nervous pathways and others, possibly by the liberation of a humoral substance.

So far as we are aware, these findings establish for the first time that electrical stimulation of the amygdaloid nuclear region can cause marked elevations of systolic and diastolic blood pressure with or without accompanying feelings of anxiety and fear and somatic motor responses. Comparable heart rate and pupillary effects have been previously reported in animals and certain emotional changes in man, by others.

A Comparison of the Cholesterol "Balance" and Nitrogen Absorption from the Human Small Intestine by an Inert Indicator Method. SAMUEL H. CHENG and MALCOLM M. STANLEY,* Boston, Mass.

A standard diet (C-250, F-103, P-116, Cholesterol-1 gram) mixed uniformly with a known amount of chromic sesquioxide (Cr_2O_3), a chemically inert and completely non-absorbable indicator, was fed to all subjects. In six patients intestinal contents were withdrawn via a tube previously introduced under fluoroscopic guidance. Terminal ileostomy discharges were collected from a seventh. The concentrations of Cr_2O_3, nitrogen, total and free cholesterol were determined in the dried food and samples of intestinal contents. The cholesterol "balance" and nitrogen absorption were calculated from the changes in the cholesterol/Cr_2O_3 and N/Cr_2O_3 ratios.

There were negative cholesterol "balances" with simultaneous absorption of nitrogen in three subjects from whom specimens of terminal ileum contents were obtained. In the subject with an ileostomy there was, during a two-day period, with 70% nitrogen absorption, a daily net loss of 163 mg. cholesterol in excess of that ingested. Net losses of 122 mg. and 258 mg. cholesterol per day occurred in two others whose nitrogen absorption averaged 40–50%.

In specimens obtained from upper small intestine (4 subjects) the cholesterol/Cr_2O_3 ratios approached twice those of food and reflected the large endogenous cholesterol secretion. Cholesterol absorption in this area was thus obscured. Nitrogen absorption was 25% or less.

The cholesterol in specimens of contents of the intestine included both the unabsorbed cholesterol from the food and from that secreted in the bile and by the intestine. The cholesterol "balance" represented the sum of the endogenous and exogenous cholesterol minus that which had been absorbed. Since the amount of endogenous cholesterol secreted could not be accurately calculated from our data, the absolute amount of cholesterol absorbed from the intestine could not be determined.

Comparative Effects of Fructose and Glucose on the Fate of Infused Amino Acids and Peptides. HALVOR N. CHRISTENSEN, PATRICIA BRYAN WILBER, BARBARA A. COYNE and HERBERT FISHER, Boston, Mass. (Introduced by Joseph M. Hayman).

The fate of infused amino acids, whether anabolic or catabolic, is known to be largely influenced by the simultaneous availability of appropriate calorie sources. Elman *et al.* recently reported that more positive nitrogen balances were obtained in surgical patients when fructose rather than glucose was included in an intravenously injected protein hydrolysate. This they attributed to the well-known rapidity of the removal of fructose from the circulation. Elman also reported lower plasma amino acid levels during the course of the fructose-containing infusions.

We have investigated the influence of the type of sugar present on the distribution and fate of amino acids and peptides infused intravenously into convalescent surgical patients and normal volunteers. In the case of a fibrin hydrolysate ("Aminosol," Abbott Laboratories, Inc.) the urinary spillage of peptides was diminished from about 30% to about 13% when 10% fructose replaced 5 or 10% glucose. The wastage of free amino acids was also substantially decreased. These effects were shown to be independent of any diuretic action of the glucose solution.

In contrast to Elman's report of lower plasma amino acid levels, in our hands fructose led to slightly higher levels in 8 comparisons in 5 subjects. His use of a modified Folin colorimetric method (strongly criticized by Chinard and Van Slyke) and our use of the manometric ninhydrin method might conceivably explain this difference.

Stronger uptake of amino acids by the cells would not necessarily favor their anabolic utilization, considering that a large part of that uptake is hepatic. An inhibition of deamination and urea formation in the presence of abundant carbohydrate may instead be the factor operating to swing the balance to the anabolic side. In that case *higher*, rather than *lower*, amino acid levels would be produced in the presence of a more adequate source of energy.

The Production of Experimental Meningitis in the Rhesus Monkey. KENNETH L. CLARK, LOUIS P. JERVEY and PARK BIEHL, Cincinnati, O. (Introduced by Morton Hamburger).

In order to test the effect of the enzymes streptokinase and streptodornase in the liquefaction of exudate in the subarachnoid space, attempts were made to produce an experimental meningitis in rhesus monkeys. The endpoint was to be an infection in which abundant exudate would be laid down but wherein the animals would live long enough so that the effect of enzymes could be studied.

Inoculations of various bacteria suspended in gelatin-Locke's solution were made into the cisternal subarachnoid space of anesthetized animals. Approximately daily cisternal taps were performed. Some of the animals were sacrificed at varying periods after infection; others died. Strains of E. coli, H. influenzae and Pseudomonas aeruginosa, from the spinal fluid of human cases of meningitis, Pneumococcus I, from the blood stream of a patient, Streptococcus viridans, from the blood stream of a case of subacute bacterial endocarditis, and a strain of Pneumococcus III were employed.

Pneumococcus I and E. coli produced meningitis, but killed the animals in 1 to 4 days. H. influenzae produced a very mild infection from which the monkeys recovered

spontaneously. Streptococcus viridans produced pleocytosis and a positive Pandy reaction, but spinal fluid cultures remained negative and there was no exudate.

The results with Pseudomonas were irregular until a technique was devised whereby the organisms were suspended in ground up jelled agar. By this means meningitis was produced in which a thick tenacious exudate was laid down within 48 hours around the base of the brain and the cerebellum, sometimes extending several centimeters down the spinal cord. Microscopically, the exudate consisted of great numbers of polymorphonuclear leukocytes, much fibrin, and large amounts of extracellular desoxyribonucleoprotein. Spinal fluid cultures and cultures of the exudate were always positive for the infecting strain of Pseudomonas. More than 90% of the animals survived 5 days or longer.

Histamincytes of the Blood—Eosinophils and Basophils. CHARLES F. CODE* and ROSS G. MITCHELL, Rochester, Minn.

This investigation was undertaken to determine whether there is a relationship between the amount of histamine and the numbers of eosinophils or basophils in the blood. The numbers of the cells and the concentration of histamine were determined quantitatively. Observations were made in dogs and human beings before, during and after the administration of cortisone.

No basophils were found in the blood of the dogs, and in these animals high concentrations of histamine occurred only in association with large numbers of eosinophils. Administration of cortisone always reduced the numbers of eosinophils in the blood, and at the same time lowered the concentration of histamine.

In human beings cortisone always caused a decline in the number of basophils as well as eosinophils. The values for blood histamine also always decreased. The numbers of basophils and eosinophils were definitely related, and they both were related to the level of histamine in the blood, indicating that they may both act as histamine carriers in the blood.

"Splanchnic Pooling" During the Hypotensive Action of Hexamethonium Bromide in the Dog. B. COMINSKY, J. R. K. PREEDY, H. O. WHEELER, R. M. HAYS and S. E. BRADLEY,* New York, N. Y.

The recent development of a method of estimating the circulating splanchnic blood volume (SBV) by determination of splanchnic vascular retention and dilution of I^{131} labelled human serum albumin (*Trans. Assoc. Am. Phys.*, **66**, 294, 1953) has made possible the evaluation of the role of the splanchnic vasculature as a "blood reservoir" during systemic hemodynamic adjustments. Hexamethonium bromide (7.5 to 10.0 mg. ion i.v.) was used as a means of producing such an adjustment in 8 anesthetized dogs (nembutal, 30 mg/kg) during the measurement of SBV, hepatic blood flow (BSP method), total blood volume (I^{131} HSA dilution), cardiac output (direct Fick) and arterial pressure (Statham transducer). Following administration of hexamethonium cardiac output decreased (15 to 76%, mean—31%) in association with a corresponding fall in blood pressure (19 to 47%, mean—36%). The total peripheral vascular resistance did not change significantly. The hepatic blood flow decreased (P less than .05, mean—21%) but not to the same extent as the blood pressure since splanchnic resistance was also lowered (P less than .05, mean—17%). Despite the reduction in inflow and possibly also diminished resistance to outflow, SBV increased in 6 of 8 animals (29 to 64%, mean—39%, P less than .02) suggesting an alteration in splanchnic vascular tone. Since total blood volume did not seem to change, the resultant "trapping" of blood in the splanchnic bed may have contributed to diminished venous return, leading to a lowered cardiac output and a fall in blood pressure.

C-14 Carboxyl Labeled Inulin as a Tracer for Inulin. E. COTLOVE, M. C. REDER, W. B. LOONEY, M. WALSER and L. W. MATTHEWS, Bethesda, Md. (Introduced by Robert W. Berliner).

Preliminary studies have been made of the physiological behavior of radioactive inulin, prepared by Drs. H. S. Isbell and J. D. Moyer of the National Bureau of Standards by cyanohydrin synthesis, with the addition of $C^{14}OOH$ groups in a ratio of about 1 to 200 fructose residues. Electrophoresis of 3% C-14 inulin in buffer and in plasma at pH 8 showed no migration of the inulin and no evidence of protein binding. Following intravenous injection in several species, the radioactivity was recovered in the urine completely within the error of counting, averaging 100.8% in 4 rabbits, 97.0% in 6 rats and was 101.8% in a dog and 99.0% in a man with metastatic carcinoma. Chromatographic analysis of the urine showed the radioactivity to be localized to the inulin spot. Radioactive inulin was given by continuous infusion in 4 rats; the renal clearance of the radioactivity and its volume of distribution in muscle were similar to those found previously with non-radioactive inulin. In one dog, 5 consecutive clearance periods obtained during continuous infusion of creatinine and C-14 inulin showed a ratio of C(Cr) to C(radioactivity) averaging 0.95, similar to results of Kennedy, Hilton and Berliner (Am. J. Physiol. **171**: 164, 1952) with non-radioactive inulin. The volume of distribution of radioactivity in the dog was 17% of body weight.

These results indicate that the C-14 inulin has the physiological behavior of non-radioactive inulin, with no evidence of plasma binding, decarboxylation or other type of metabolism. This compound should be useful particularly in studies requiring analysis of inulin in low concentration in the presence of a chemical chromogen blank. Further investigation is in progress.

The Effect of Growth Hormone on the Amino Acid Pool and Protein Synthesis Rate in a Pituitary Dwarf. K. R. CRISPELL, WILLIAM PARSON* and GUY HOLLIFIELD, Charlottesville, Va.

The urea space, the size of the amino acid pool, and the rate of protein synthesis have been determined in five

healthy adult volunteers using N-15 glycine and N-15 urea by the method of San Pietro and Rittenberg.

The same type of study has been carried out in a 20 year old dwarfed, sexually immature male who had undergone pituitary surgery at the age of eight. The patient was studied under the usual standard metabolic regimen. Before the administration of growth hormone (Raben) the patient was found to have an amino acid pool of 3 mg./Kg. body weight as compared to 14 mg./Kg. (range 11 to 18) in the healthy volunteers. The rate of protein synthesis was 290 mg./Kg. per 24 hours as compared to 810 mg./Kg. per 24 hours (range 610 to 1170) in the healthy volunteers.

The patient was then given purified growth hormone (Raben), 20 mg. every six hours intramuscularly and the studies repeated on the sixth day of administration. Classical balance studies on this date showed minimal nitrogen retention. The urinary nitrogen averaged 5.0 Gms. daily for the six days previous to growth hormone, and 4.4 Gms. daily for the six days while receiving growth hormone. There was, however, a profound change demonstrated by the isotope technique. The amino acid pool increased from 3 mg./Kg. to 11 mg./Kg. and the rate of protein synthesis from 290 mg./Kg. per 24 hours to 1,180 mg./Kg. per 24 hours.

This technique appears to be more sensitive than classical nitrogen balance studies in assaying changes in protein metabolism. It may also offer some insight into the mechanisms occurring with hormonal manipulation.

The Comparative Effect of Platelets on Prothrombin Utilization from Dogs in the Degenerative and Regenerative Phase of Irradiation Bone Marrow Aplasia. E. P. CRONKITE and G. J. JACOBS, Bethesda, Md. (Introduced by L. M. Tocantins).

A quantitative relationship between platelet concentration and prothrombin utilization in plasma of normal dogs has been shown by Buckwalter *et al.* and in whole blood of irradiated dogs by Jackson *et al.* In the latter studies, it appeared that the irradiated platelets were less effective than normal platelets in accelerating prothrombin conversion. In contrast the platelets in whole blood of dogs in the regenerative stage appeared more effective than platelets in the degenerative stage (Cronkite *et al.*). These phenomena might be explained on the basis of changes in the platelets or changes in concentration of plasma anticoagulants (antithromboplastins).

To investigate these phenomena, the influence of the following on prothrombin utilization was studied:

1. The comparative effect of platelets in whole blood in the degenerative stage with platelets in the regenerative stage.

2. The comparative effect of platelets in plasma from normal dogs and in plasma from dogs in the degenerative phase by varying platelet count with differential centrifugation and silicone technique without anticoagulants.

The following relationships were demonstrated:

1. At all platelet levels, platelets in the degenerative phase of radiation injury were less effective in accelerating prothrombin conversion than platelets in the regenerative phase when the conversion took place in whole blood.

2. In plasma at similar platelet levels, platelets of irradiated dogs in the degenerative phase were less effective than normal platelets.

3. In both cases above, platelets in the degenerative phase were less effective by a factor of two or three.

4. Technically, it was not possible to cross-transfer irradiated or normal platelets to the opposite plasma and obtain meaningful results. It was therefore not possible to ascribe these alterations in platelet behaviour primarily to a plasma constituent or to qualitative changes in the platelet but previous work by Tocantins strongly suggests that lipid antithromboplastins may partly contribute to the lesser effectiveness of platelets in the degenerative stage.

The Renal Oxygen Consumption in Diabetic Nephropathy as Related to Tubular Functional Mass. ARCHER P. CROSLEY, JR., GEORGE G. ROWE, JOHN H. HUSTON, GEORGE M. MAXWELL and CHARLES W. CRUMPTON, Madison, Wis. (Introduced by Edgar S. Gordon).

The ratio of renal oxygen consumption (total renal blood flow × arterial-renal venous oxygen difference) to renal tubular mass (Tm_{PAH}) in 4 patients with diabetic nephropathy has averaged 0.659. This value represents a threefold increase from that observed in normal patients (0.232). The specificity of this elevation to diabetic nephropathy is reflected in further studies indicating that it is significantly different from results obtained in patients with essential hypertension (0.216), labile diabetes without nephropathy (0.217), and the presence of poor renal function (0.070).

These results demonstrate a unique renal metabolic pattern in diabetic nephropathy which may be related to other endocrine abnormalities associated with this condition.

Homeostatic Limits in Patients with Chronic Nephritis and Therapeutic Implications. JOHN D. CRAWFORD, GERALD A. KERRIGAN, WINSTON E. COCHRAN, MARY TERRY and NATHAN B. TALBOT,* Boston, Mass.

Studies have been performed in a small series of patients with advanced chronic nephritis to define their residual ranges of renal capacity for homeostatic adjustment. The study technique involved altering rate of input of selected individual constituents of intake to provide challenge situations. In such circumstances the adaptive behavior of the patients has been described and limits of renal capacity for homeostatic adjustment have been taken as those points at which further compensatory changes in urinary excretion fail to occur while pathologic deviation in body content or concentration of the constituent under study develops. Limits have been described for urine water concentration and volume, pH and ammonium ion content and for the excretion rates of sodium, potassium, phosphate and urea.

These studies have indicated certain important differences in the impairments shown by individual patients.

They have shown major reductions in capacity to vary urine water concentration. Urine volumes greater than thirty per cent of the glomerular filtrate may be formed. Renal ability to conserve sodium and potassium has been found unimpaired except in acidosis when fixed cation losses result from reduced excretion capacity for hydrogen and ammonium ions. Maximum excretory capacities for potassium and phosphate are diminished in proportion to glomerular filtration, but their excretion, like inulin and urea clearances, may be augmented by large sodium intakes.

The definition of these residual renal capacities has facilitated design of individualized diets which permit restoration and maintenance of normal body composition. This has been followed by changes including improvement in well-being, resumption of growth, healing of rickets and reduction of hypertension. While the degree of alteration in ultimate prognosis referable to such therapy is still uncertain, the results obtained to date suggest that this method of approach may prove both of practical and basic interest.

The Visceral Circulation in Patients with Coarctation of the Aorta. JAMES W. CULBERTSON,* WALTER M. KIRKENDALL, JOHN W. ECKSTEIN and GEORGE N. BEDELL, Iowa City, Ia.

Available reports on renal and total systemic (= pulmonary) blood flow in patients with coarctation of the aorta are conflicting and often fragmentary. Hepatic (= splanchnic minus renal and adrenal) circulation remains unrecorded.

We have studied renal circulation in 14 patients, 10 of them having cardiac output determinations also, and 8 of these having additional hepatic blood flow estimations. Standard hemodynamic (Fick principle) and plasma clearance chemical methods were employed, including direct registration of intra-arterial pressure levels from both brachial and femoral arteries.

Seven patients had elevated cardiac indices (4.12 – 5.56 L/min./M^2), 1 a normal value (3.04) and 2 in congestive myocardial decompensation subnormal values (2.24 and 1.56). Renal plasma flow was elevated (971 ml/min.) in 1, normal (553 – 697) in 8, and subnormal in 5 (of whom 3 were in heart failure). Only these 5 had increased renal vascular resistance. Hepatic hyperemia was found in 3 (2037 – 2559 ml/min.), high normal blood flow (1545 – 1866) in 3, and low normal (1145 – 1186) in 2 who were in subclinical heart failure. Splanchnic vascular resistance was normal in these 2 but reduced in 6. Bromsulfalein plasma clearance (C_{BSP}) was normal (441 – 757 ml/min.) in 7, low (142) in 1 and elevated (964) in 1. Splanchnic oxygen consumption (85 – 113 ml/min.) averaged above normal, as did total oxygen consumption. Systolic arterial hypertension was found in the upper compartment and normal mean arterial pressure in the lower.

The data fail to show significantly increased arteriolar resistance locally or generally, diastolic hypertension, or renal ischemia in patients not in myocardial decompensation. They do support normal renal function, hepatic hyperemia with decreased splanchnic arteriolar resistance, and increased total blood flow and general and splanchnic metabolism. This is in sharp contrast to the hemodynamics of essential arterial hypertension.

Comparative Absorption Rates in the Upper and Lower Human Small Intestine. ALVIN J. CUMMINS and RAINE JUSSILA, Philadelphia, Pa. (Introduced by Thomas E. Machella).

Data from animal experiments comparing absorption rates in various levels of the small intestine are meager and conflicting. Similar data are not available for the human. Because of the possible significance of such knowledge in various clinical conditions, comparison studies of absorption rates in man were undertaken.

The intubation technic of Nicholson and Chornock was used, permitting measurement of the absorption rate of a 45 cm. segment of the small bowel. In the present study, rate of absorption in the upper intestine was compared with that in the lower ileum in the same patient, the tests being run consecutively. Glucose solutions in concentrations of 5% and 10% were used as test substance in seven and six normal subjects, respectively. Urea absorption from solutions of similar tonicity was also determined in three subjects.

The resulting data indicate a mean absorption rate in the ileum 29% below that of the upper intestine for 5% glucose and 46% less for 10% glucose. Reduced absorption rates of similar magnitudes in the lower bowel were also demonstrated for urea. The results further suggest that the absorption rate for water may be reduced in the ileum, as indicated by the larger volumes of fluid aspirated there.

Failure to demonstrate significant difference in the concentration of unabsorbed glucose aspirated from either site implies that reduced ileal absorption rate is not due to greater dilution. Likewise, comparable absorption rates of glucose and the readily diffusible substance urea make it unlikely that the observed difference is due to variation in mucosal phosphorylating capacity. Balloonkymographic studies during the performance of the tests confirm the previously observed gradient of lessened motility from upper to lower intestine and this motor difference may possibly be implicated in the variation in absorption rate.

Observations on Spontaneous and Experimental Cheyne-Stokes Breathing in Man. JAMES H. CURRENS and RHETT TALBERT, Boston, Mass. (Introduced by Mandel E. Cohen).

Cheyne-Stokes breathing is characterized by recurring periods of apnea and subsequent respirations which wax and wane. A systematic study of this phenomenon has been in progress which has endeavored to (1) record the respirations, (2) study arterial blood chemistry in different phases, (3) evaluate cardiac, neurological and mental status and (4) test the effect of drugs and stimuli.

Eleven patients have been observed to have spontaneous

or induced Cheyne-Stokes breathing. The breathing was recorded in five patients while inhaling 100 per cent oxygen, and in one patient Cheyne-Stokes breathing was observed with a persistent blood oxygen saturation of 100 per cent. Stimuli such as pain, noise, attention, increased awareness and carbon dioxide have all been found to alter or dispel the Cheyne-Stokes breathing. Conditions which have enhanced this breathing have been relaxation and sleep. In three patients this breathing occurred after the lowering of blood pressure from protoveratrine or hexamethonium. Aggravation of abnormal neurological signs was observed concomitantly with Cheyne-Stokes breathing. The constant dispelling of this phenomenon with carbon dioxide inhalation has been striking; this is contrasted with the fact that oxygen usually had no effect.

Nine cases have had abnormal neurological signs or a history of apoplexy. Four autopsied cases have each demonstrated cerebral injury, usually bilaterally. One of the autopsied cases had no abnormal neurological signs.

It is concluded that emphasis should be placed upon the neurological aspect of this type of breathing rather than on blood chemical changes or degree of congestive heart failure. The first description of this disorder by Cheyne in 1818 was a cardiac patient who experienced apoplexy which rendered him "speechless and hemiplegiac" and whose breathing "would entirely cease for a quarter of a minute then by degrees become heaving and quick."

Inoculation Syphilis in Man. JOHN C. CUTLER, LOPO DE MELLO, BERNARD I. KAPLAN, HAROLD J. MAGNUSON,* SIDNEY OLANSKY and EVAN W. THOMAS, Chapel Hill, N. C.

Sixty human prisoner volunteers with well documented medical histories were inoculated intracutaneously with the Nichols strain of *T. pallidum*. The experiment was designed to assess immunity in relation to serologic tests with lipoidal antigens, treponemal immobilization test, and duration of original infection prior to treatment. Eight of the subjects were non-syphilitic, the remainder previously had had syphilis and all except 4 had been treated for the disease. Prior to challenge inoculation one half the group received 5×10^7 heat killed *T. pallidum* intracutaneously and subcutaneously. An anamnestic T. P. I. response was demonstrated in some patients, but reagin titers were unchanged. Graded inoculums ranging from 10 to 10^4 virulent organisms were inoculated intracutaneously at 4 sites in each of the controls. The 50% infectious inoculum was found to be between 10 and 10^2 organisms. All other subjects were challenged at a single site with 10^5 organisms.

The early course of the disease in the normal controls was analogous to that observed in naturally acquired infection as measured by clinical and serologic changes. Of 11 patients previously treated for early syphilis, 9 developed dark field positive lesions, 2 dark field negative lesions. All showed increasing titers of reagin and T. P. I. antibody. Of 34 patients previously treated for late syphilis, 2 developed dark field positive lesions, 12 dark field negative lesions with increasing serologic titers, 3 dark field negative lesions without serologic change, and 16 showed no clinical or serologic evidence of infection. Two of the lesions were clinically gummata. None of the 4 untreated patients showed clinical or serologic response to inoculation. One patient previously treated for asymptomatic neurosyphilis had as sole response a redevelopment of active asymptomatic neurosyphilis. This patient represents the only reinfection in which no cutaneous lesion developed at site of inoculation. All patients were treated with penicillin no later than 4 months after inoculation.

Acquired immunity in human syphilis is shown to exist and appears to be related to the duration of the original immunizing infection prior to its treatment.

The Character of the Records Obtained from the Aperiodic Ballistocardiograph and Their Relationship to Those from the High-Frequency Bed (Starr) Ballistocardiograph. D. C. DEUCHAR, S. A. TALBOT, F. W. DAVIS, JR. and W. R. SCARBOROUGH, Baltimore, Md. (Introduced by Benjamin M. Baker, Jr.).

Analysis of the high-frequency bed ballistocardiograph has demonstrated defects of this method of recording motions of the body resulting from cardiovascular forces. Important amongst these is the excitation of the spring and damper system formed by the dorsal tissues of the subject, resulting in a distorted frequency response in the ballistocardiographic range.

A light platform floating on mercury has been constructed and ballistocardiograms have been recorded using an accelerometer. This system has no restoring force, so is aperiodic, and has minimal damping. These properties minimize excitation of the dorsal tissues and approximate the ideal of a freely floating body. Acceleration records so obtained measure forces referred to the body without distortion through most of the ballistocardiographic spectrum now known. Mathematical analysis suggests that displacement records from the high-frequency bed represent grossly the slower forces acting on the body, so the two types of records can justifiably be compared to study the distortion present in the latter.

Records of 60 subjects, normals and abnormals, have been obtained from both systems. Subjects with normal high-frequency bed records yield a consistent pattern on the aperiodic bed which contains slurs, splinters and faster detail, with the major waves slightly preceding the high-frequency bed record in time; late systole typically shows a series of small forces, without a major footward contribution, in place of the K wave which we therefore regard as artefact, in normal records, due to after-oscillation. Diastole also shows consistent well-defined forces. Abnormal records demonstrate well a similar, consistent relationship to the high-frequency bed record in all cases. This supports the view that the high-frequency bed displacement ballistocardiograph reflects grossly the force on the body but significantly distorts its detail both in timing and in wave form.

Erythrocyte Metabolism. Studies on the Metabolic Behavior of Reduced Glutathione in Human and Avian Erythrocytes. ELHANAN DIMANT, EDITH LANDSBERG and IRVING M. LONDON,* New York, N. Y.

In studies on the ageing process in erythrocytes, the patterns of metabolic behavior of various constituents of the erythrocytes are under investigation. This report is concerned with reduced glutathione, which has been implicated in the maintenance of integrity of the structure of the erythrocyte.

Glycine labeled with N^{15} was administered to a normal man, and glutathione and hemin were isolated from the erythrocytes at intervals thereafter. The isotope concentrations in the hemin reveal a normal pattern of survival of erythrocytes. The isotope concentrations in the reduced glutathione indicate that the nitrogen, derived from glycine, of the reduced glutathione in the human erythrocyte is in the dynamic state with a half-time value of approximately four days.

Incubation, *in vitro*, of intact or lysed human or duck erythrocytes with 2-C^{14}-glycine results in rapid incorporation of the labeled carbon in the reduced glutathione of the erythrocytes. These findings indicate the existence of a system in the mature erythrocyte for continued replacement of the glycine moiety of reduced glutathione and provide a basis for the study of the origins, and the mechanism of reduction, of glutathione of erythrocytes.

The Effect of Tetraethylammonium Chloride on the Pressor Response to Epinephrine and Norepinephrine. R. J. DISALVO, JAMES D. EVANS and ALBERT A. BRUST,* Atlanta, Ga.

The phenomenon of potentiation of the pressor effects of epinephrine, norepinephrine and other pressor substances during autonomic blockade with tetraethylammonium chloride (TEAC) has been extensively studied in animals. In view of the lack of similar controlled studies of this phenomenon in man, this study was undertaken in order to determine whether similar responses occurred in human subjects, since such responses may be a factor in the pressor response to TEAC in pheochromocytoma, and to aid in clarifying the interplay of neurogenic and humoral factors in the regulation of vascular tone.

Comparative studies of the blood pressure and pulse effects of rapid intravenous injection of TEAC before and during constant intravenous administration of l-epinephrine and l-norepinephrine have been made in six normotensive subjects. Blood pressure was measured by the auscultatory method; in 3 subjects direct arterial pressures were also recorded from a brachial artery by a Statham pressure transducer and strain gauge amplifier.

During a control period, a rapid injection of 400 mgm. of TEAC was given, intravenously. When the effects of the drug disappeared, intravenous infusion of .085 mcg./Kg./min. of epinephrine was begun. During the infusion, when blood pressure and pulse stabilized, 400 mgm. of TEAC was again given intravenously. In the same subjects on a subsequent day, the procedures were repeated except that .085 mcg./Kg./min. of norepinephrine was given.

In all subjects a depressor response to TEAC occurred in both control periods (mean $-22/-12$). The response to TEAC remained depressor, although of less magnitude, during epinephrine infusion. During norepinephrine infusion, a pressor response to TEAC occurred in all subjects (mean $+20/+12$), and the bradycardia due to norepinephrine was abolished.

The results indicate that in the range of the physiologic doses used, TEAC augments the pressor effects of norepinephrine, but not of epinephrine, and suggest that the mechanism of the pressor response to TEAC in pheochromocytoma may be due to the presence of circulating norepinephrine.

The Exophthalmos-producing Substance in the Serum of Patients Suffering from Progressive Exophthalmos. BROWN M. DOBYNS* and LOIS A. WILSON, Cleveland, O.

It has been known for a long time that crude anterior pituitary extracts will produce both thyroid hyperplasia and exophthalmos when injected into the animals. We have recently shown that the exophthalmos producing substance (EPS) is different from the thyroid stimulating hormone. By chemical methods these two substances have been separated and their actions independently demonstrated.

Fundulus, an Atlantic minnow, has proven to be an excellent test object for the exophthalmos producing substance. Amounts of dried extracts as small as 25 micrograms will cause a positive exophthalmic response in these fish. The response usually can be recognized grossly in 3 to 6 hours, but it may be accurately determined by measuring the intercorneal distance in each fish before and following administration of the test material.

After testing whole serum from many patients with severe or progressive exophthalmos in the past with only borderline or negative responses, we have recently observed that the serum of 3 such patients caused a positive response when administered to the fish.

In contrast to the prompt response described above, the response from whole serum usually required several days. Because the volume of fluid that can be injected into the coelomic cavity of these fish is small, 3 injections of 0.25 ml. of serum are given over a period of 24 hours. Some of our previous failures to demonstrate an exophthalmos producing substance in whole serum have probably been due to 1) an insufficient period of observation following injection, 2) insufficient amounts of whole serum given (single doses), and 3) marked variation in responsiveness of lots of fish throughout the year. Control observations, using normal saline, normal saline containing a standard active EPS pituitary preparation and pooled normal serum, have been made.

Resistance to Airflow through the Tracheobronchial Tree as Measured by Means of a Body Plethysmograph. A. B. DuBois, S. Y. Botelho and J. H. Comroe, Jr.,* Philadelphia, Pa.

Since the ratio of pressure/flow measures resistance, in order to obtain resistance to airflow through the tracheobronchial tree, it is necessary to have simultaneous measurements of airflow and alveolar pressure during the period of flow. This is accomplished by having a subject sit in an airtight body plethysmograph and breathe air from the plethysmograph through a flowmeter. Pressure changes in the plethysmograph are measured continuously during the respiratory cycle and represent alveolar volume changes caused by compression or decompression of the alveolar gas during expiration and inspiration. For example, for gas to flow out of the alveoli during expiration, the alveolar pressure must rise above atmospheric; if at any one instant the absolute alveolar gas volume is 3000 ml and the alveolar pressure rises to 10 cm H_2O, the change in alveolar gas volume, due to compression, is 30 ml. Since such small changes in alveolar gas volume during airflow can be detected by the change in pressure in the body plethysmograph and one other unknown, the absolute alveolar gas volume can be obtained by measuring simultaneous change in alveolar volume and pressure in the tracheobronchial system during the period when flow has been interrupted by a shutter, alveolar pressure during airflow can be calculated. Measuring simultaneous alveolar pressure and airflow in this manner, we have found the resistance of the tracheobronchial tree to airflow to be 1.63 cm H_2O/L./sec. ± S.D. 0.36 in 15 normal subjects. In subjects with increased airway resistance, the values are considerably higher.

Metabolism of Compound S in Rheumatoid Arthritis; Effect on 17-Ketosteroid Excretion. Ivan F. Duff, William D. Robinson, William M. Mikkelsen and Aletha M. Hemerline, Ann Arbor, Mich. (Introduced by J. W. Conn).

Recent studies (J. Lab. & Clin. Med., Jan., 1954) by Conn, Fajans, Louis, Seltzer suggest that the excretory level of 17-ketosteroids can be modified by altered metabolism of adrenocortical steroids, and disclosed that patients with liver disease excrete a much smaller proportion of administered Compound S as 17-ketosteroids than do normal individuals. In normal subjects, on the second consecutive day of Compound S administration orally in doses of 400 mgm. daily, there is a peak increase in urinary 17-ketosteroid excretion of 25 to 45 mgm. above baseline values.

This test has been applied to 6 men and 6 women with rheumatoid arthritis. Baseline excretion of 17-ketosteroids was below normal in 10 of these 12. In only 4 patients did oral administration of compound S produce a normal increment in 17-ketosteroid excretion (23 to 73 mgm.); in one subject the response was 17 mgm. In the remaining 7, the rise in excretory level was 12 mgm. or less, with no increase whatsoever in 2 subjects. Further alteration in excretory pattern characterized by delay in peak values was seen in 5 patients. Liver function, as measured by serum bilirubin, urinary urobilinogen and bromsulphophthalein retention, was normal in these patients, with the exception of excessive BSP retention in one. The abnormal serum protein pattern commonly observed in rheumatoid arthritis was present in 8. There was no obvious correlation with duration, severity or extent of the disease process.

These preliminary results suggest that further investigation of the metabolism of adrenocortical steroids in rheumatic and other chronic diseases may be informative.

Some Differences in Ventricular Contraction and Relaxation in Patients with Mitral Stenosis and Mitral Insufficiency as Studied by the Kinetocardiogram. E. E. Eddleman, Jr. and Robert H. Yoe, Jr., Birmingham, Ala. (Introduced by T. R. Harrison).

Low frequency precordial chest movements (kinetocardiograms) were recorded in patients with mitral stenosis and mitral insufficiency by means of a bellows system previously described. The events were correlated with the electrocardiogram, carotid pulse, and ballistocardiogram.

Patients with mitral stenosis revealed a marked anterior or outward movement of the precordium occurring before ejection which was sustained above the baseline during systole. This was followed by an inward movement during isometric relaxation. In contrast to this, patients with mitral insufficiency had an inward movement of the chest during early ejection followed by a large outward movement just before the carotid incisura.

The findings were interpreted as indicating an exaggerated anterior swing of the heart during systole in mitral stenosis probably as a result of the contraction of the right ventricle. The swing predominates during ejection. However, as the right ventricle releases, the left ventricle pulls the heart posteriorly and leftward in the chest. Mitral insufficiency appears to lack the early marked anterior swing but has a late systolic swing of the heart. Thus, there appear to be fundamental differences in ventricular contraction and relaxation in patients with mitral stenosis and mitral insufficiency.

The Effect of Myocardial Anoxia on Myocardial Metabolism in Vivo. Sterling Edwards, A. Siegel and Richard J. Bing,* Birmingham, Ala.

The effect of myocardial anoxia resulting from hemorrhagic shock was studied in 16 anesthetized dogs. Shock was produced with a modified technique of Wiggers (blood pressure reduction to 50 mm. Hg for 90 minutes, then 30 mm. Hg for 60 minutes, followed by reinfusion of blood). Coronary blood flow and myocardial extraction and usage of oxygen, glucose, pyruvate and lactate were determined during the control period and during the oligemic and normovolemic phase. During the oligemic phase, coronary flow, coronary vascular resistance, myocardial oxygen consumption and mechanical efficiency were reduced. Reinfusion of blood did not result in a

return of myocardial oxygen consumption to normal control values. The most significant observation was a reversal of the usual positive pyruvate balance during the oligemic phase with the concentration of pyruvate in coronary vein blood significantly exceeding that in coronary artery blood. This was accompanied in some instances by similar reversal in the extraction of a reducing substance determined as glucose by the micromethod of Hagedorn. Myocardial oxygen extraction of lactate remained positive throughout. These results indicate that reduction of coronary blood flow results in severe myocardial anoxia which may be responsible for (1) inhibition of enzyme systems engaged in the breakdown of pyruvate (cocarboxylase); (2) increased glycolysis, which may represent the prime source of cardiac energy under those conditions. As a result, pyruvate accumulates. Continued extraction of lactic acid might result from conversion of lactate to pyruvate or its conversion to carbohydrate intermediates in the direction of glycogen. These metabolic changes point to the possibility of an anaerobic metabolism of heart muscle in vivo.

The Effect of Congestive Heart Failure on Total Blood Volume, Red Cell Mass, and Plasma Volume as Measured by Radioactive Chromium-Labeled Red Cells. SEYMOUR EISENBERG, Dallas, Tex. (Introduced by Elias Strauss).

The effect of congestive heart failure on total blood volume and circulating red cell mass remains a matter of considerable controversy. Measurements with T-1824 dye have yielded consistently increased values but it is likely that they included an expanded lymphatic space. Measurements utilizing radiophosphorus have yielded inconsistent changes, but the instability of the P-32 red cell complex precludes satisfactory equilibration periods.

Determination of blood volume and circulating red cell mass with radiochromium labeled red cells suffers from neither of these defects. This method has therefore been utilized in the study of 27 subjects with congestive heart failure. Comparisons were made between pre-treatment and post-treatment values and between these and the values of 20 normal subjects. The patients were grouped according to the "degree' of congestive failure, dependent upon duration, severity, and tractability.

Congestive heart failure resulted in a 22% increase in blood volume, a 25% increase in red cell mass and a 17% increase in plasma volume. There was a 38% increase in blood volume in the "severe" group as opposed to a 14% increase in the "mild" group. After treatment, the blood volume decreased in 50% of the patients. This shrinkage involved a reduction in both red cell mass and plasma volume; only rarely did selective shrinkage of plasma volume occur. The magnitude of hypervolemia was correlated with the degree of cardiomegaly; following treatment, shrinkage of blood volume tended to parallel the decrease in heart size.

It is concluded that:

1. Heart failure is usually accompanied by an expanded blood volume, which shrinks after compensation is restored.
2. The expanded blood volume, as well as the reduction following compensation, involves red cells and plasma equally.
3. Changes in blood volume parallel changes in heart size and are not apparently related to the formation or delivery of edema.

The Effect of Variations in Amino Acid Intake and Protein Deficit on the Metabolic Response of Soft Tissues and Bone to Cortisone Acetate. LEONARD P. ELIEL* and ROBERT P. HEANEY, Oklahoma City, Okla.

The effects of variations in protein anabolic rate on the metabolic response to oral cortisone acetate, 200 to 600 mg./day, have been studied in a patient who was fed synthetic isocaloric diets of differing amino acid content.

On a zero amino acid intake, after urinary nitrogen excretion had stabilized at about 2.0 gm./day, administration of cortisone resulted in a transient, slight increase in nitrogen excretion, averaging 0.1 gm./day. Amino acids 50 gm./day, and cortisone, were given during a subsequent period of marked positive nitrogen balance. A slight negative balance was achieved only on high doses of cortisone. With an amino acid intake of 100 gm./day, after establishing nitrogen equilibrium, cortisone resulted in a prompt and sustained negative nitrogen balance, averaging 1.5 gm./day.

Urinary calcium, during administration of cortisone, rose 96 and 173 mg./day, at the zero and 50 gm. amino acid levels, respectively. Preexistent hypercalcuria confused interpretation of a rise at the 100 gm. level. The serum alkaline phosphatase concentrations fell significantly at all amino acid levels when cortisone was exhibited.

Retention of sodium, chloride and water, and eosinopenia, were manifested at all amino acid levels, and independently of the nitrogen balance, during administration of cortisone.

The results provide further evidence that the principal effect of cortisone on protein metabolism is antianabolic. However, in the presence of a strong anabolic stimulus (protein deficit plus adequate amino acid intake), administration of cortisone failed to induce nitrogen losses, except in very high doses. The increases in urinary calcium, during administration of cortisone, indicated either that bone anabolism continued, to a small degree, on an amino acid-free diet, and was inhibited, or that cortisone also stimulated bone catabolism.

Plasma Mixing and Removal Curves for Fe^{59} and I^{131} After Intravenous Injection of Doubly Labeled Iron Binding Globulin. P. J. ELMLINGER, S. P. MASOUREDIS, J. SONI, G. E. FULTON and S. L. BELKNAP, Berkeley, Calif. (Introduced by John H. Lawrence).

Studies of iron turnover involving the labeling of total plasma iron have aroused interest in the metabolic relationship between the circulating plasma iron (CPI) and its globulin carrier. The turnover rate of the iron binding

globulin (IBG) as well as the plasma iron turnover rate was determined with Fe^{59} globulin in which the protein was trace labeled with I^{131}.

In patients given the doubly labeled IBG the two isotopes in the serial samples were separated chemically prior to radioassay. IBG singly labeled with I^{131} was used in some patients for comparison of IBG turnover rates with iron turnover rates determined several months earlier.

Extrapolation to zero time of the first post-mixing component was used for defining space of dilution of Fe^{59} and I^{131} IBG. Turnover rates of iron and of the IBG were determined from their respective half-times, the concentration, and volume.

The serial changes in body distribution of radioactivity after injection of IBG labeled only with I^{131} are similar to those observed with gamma emitters confined within the vascular bed and are in contrast to the changes which occur during the first three weeks following the injection of IBG labeled with Fe^{59}. It was calculated that 0.1 to 0.2 of the total IBG was replaced per day which differs markedly from the replacement rate of the total iron.

The above data are compatible with the concept that the globulin functions as a carrier for iron. In addition, the data suggest that some abnormalities in the sideroKinetic pattern in various disease states are reflections of disturbed carrier globulin metabolism.

Studies of the C-Reactive Protein in the "Post-Commissurotomy" Syndrome. SAMUEL K. ELSTER and HARRISON F. WOOD, New York, N. Y. (Introduced by Alexander B. Gutman).

The "post-commissurotomy" syndrome developed in eight of thirteen patients with rheumatic mitral stenosis two weeks to seven months after commissurotomy. The characteristic chest pain, fever and cough, often with signs of pluritis and pericarditis, usually lasted 1–3 weeks. Multiple attacks occurred in 3 patients.

The C-reactive protein (CRP) in the sera of twelve patients was determined pre- and post-operatively. The CRP became positive within 24 hours post-operatively and remained elevated 1–8 weeks. Markedly elevated titers (4–6+) occurred in ten patients. Control studies in seven patients following surgery for congenital heart disease revealed similar high post-operative titers of CRP but disappearance within a week in six.

Of the eight patients with the "post-commissurotomy" syndrome, four had transiently positive CRP pre-operatively; three had auricular biopsies positive for "Aschoff bodies." Of the remaining five patients, one had a positive CRP pre-operatively and two had positive auricular biopsies.

Serial determination of the CRP was performed in six instances of the "post-commissurotomy" syndrome. In all, the CRP became positive at onset and persisted throughout the period of clinical activity. As the syndrome remitted, the CRP diminished and in four instances persisted for variable periods following apparent clinical remission. In three cases, salicylates appeared to suppress the clinical manifestations. In one, this was associated with rapid subsidence of the CRP. Following withdrawal of the salicylates, the CRP reappeared promptly and without any further treatment slowly subsided.

The consistent appearance of CRP in the "post-commissurotomy" syndrome does not indicate that this disease necessarily represents a manifestation of rheumatic fever. The determination appears to be useful in the diagnosis and management of the "post-commissurotomy" syndrome.

Studies of an Unusual Patient with Plasmacytosis. RALPH L. ENGLE, JR. and LILA A. WALLIS, New York, N. Y. (Introduced by David P. Barr).

A 38 year old man was found to have 40% plasma cells in the bone marrow and an occasional one in the peripheral blood. Almost all of the cells contained rod- or rectangular-shaped cytoplasmic inclusion bodies which took the azur stain. The serum protein was 5.4 gm.% with an A/G ratio of 4.1/1.3. Filter paper electrophoresis revealed a marked hypogammaglobulinemia. The urine contained large quantities of a protein resembling Bence-Jones protein which precipitated out upon heating and redissolved upon boiling. This protein migrated as beta-globulin on filter paper electrophoresis. There was hepatosplenomegaly and a moderate anemia. X-rays of the bones showed minimal osteoporosis. Two small rib fractures were present. A diagnosis of multiple myeloma was entertained.

There were, however, other features not readily explained by the diagnosis of multiple myeloma, but more characteristic of the adult Fanconi syndrome. Among these was a constant aminoaciduria, measured by two-dimensional paper chromatography, which was not found in 11 patients with multiple myeloma. In addition, glycosuria of renal type, hypocalcemia, hypophosphatemia, and an elevated alkaline phosphatase were found.

The urinary protein differed from the form described by Bence-Jones in that at pH 5 it precipitated out at 65°C rather than at 45–55°C. Studies of the amino acid composition of this protein by means of two-dimensional paper chromatography revealed the presence of methionine in considerable amounts in contrast to typical Bence-Jones protein which in our experience and that of others contained practically no methionine.

The Serum Precipitable Iodine (SPI) in Surgical Stress and Serious Illness. WILLIAM W. ENGSTROM and BLANCH MARKARDT, Milwaukee, Wis. (Introduced by Randall G. Sprague).

The following studies were undertaken to determine whether surgical stress elicits effects which alter thyroid function (as determined by the SPI) and to define more clearly the limits of the SPI as a diagnostic technic. In 21 of 24 operated patients no significant change in the SPI occurred either acutely (hours) or during one week after surgery. Thus the phenomena associated with surgical stress do not appear to be directly related to al-

terations in the concentration of thyroid hormone in serum. However, the SPI progressively fell to abnormally low levels in three patients who expired 5, 6 and 19 days post-operatively.

The behavior of the SPI was then studied in 32 seriously ill patients who suffered from a variety of conditions. Fourteen of these expired; the remainder survived during the period of observation. The SPI varied from 1.2 to 5.9 micrograms % (mean: 3.2 micrograms); these values contrast sharply with our control range of 3.5 to 7.0 micrograms % (mean: 5.1 micrograms). The abnormally low values appeared to be related to some phenomenon of the illness itself rather than to survival or to acute stress. Similar to the findings of Recant and Riggs (J. Clin. Investigation **31**: 789, 1952) in patients with nephrosis, we found a close correlation between the SPI and the level of the serum albumin. However, we do not have data to support the idea that hypoalbuminemia from any cause is invariably associated with low SPI. Yet, it seems that in many illnesses, especially if severe, chronic and associated with hypoalbuminemia, the concentration of the SPI decreases. It is not entirely clear what this phenomenon signifies in terms of the physiology of the thyroid hormone, but it emphasizes that caution must be exercised in the interpretation of low values for SPI.

Spectrophotometric Determination of Streptolysin-O Activity and Inhibition. HARRY A. FELDMAN * and ANNE L. GILLEN, Syracuse, N. Y.

Herbert in 1941 reported that the activity of streptolysin-O and the concentration of antistreptolysin-O could be measured with greater accuracy if 50% rather than 100% end-points were used and that saline phosphate buffered at pH 6.5 and rabbit erythrocytes in approximately 2.5% concentration were advantageous. Liao later described a modification of the antistreptolysin-O test using 50% end-points and rabbit erythrocytes in 5% concentration. Since the 50% unit should be superior to the currently favored 100%, this study of the conditions required to define a reproducible $SL_{H_{50}}$ unit was undertaken. Sheep erythrocytes stored in modified Alsever's solution were used and initially veronal bicarbonate buffered saline (VBBS) at pH 7.3–7.4 was the diluent; all readings were made with the Coleman Junior spectrophotometer at wavelength 550 mμ and plotted by reference to the Von Krogh equation. It was demonstrated that phosphate buffered saline was preferable and that pH 7.1–7.2 was superior to 6.5. With the VBBS, streptolysin-O activity was significantly reduced during incubation but this could be prevented by including glycerine in the mixture or by using phosphate buffer. The $SL_{H_{50}}$ of different lots of streptolysin-O determined in this way did not vary significantly. In contrast to other reports, the relationship between the $SL_{H_{50}}$ unit and the concentration of erythrocytes is not linear except at erythrocyte concentrations above 3%. In the range 1–3%, the $SL_{H_{50}}$ unit is relatively constant. Sheep erythrocytes can be substituted satisfactorily for human erythrocytes if allowance is made for the difference in erythrocyte size. The former are approximately 64% as large as human erythrocytes; thus a 4.8% suspension of sheep erythrocytes is numerically equivalent to a 7.6% suspension of human cells. From these findings a test for the measurement of antistreptolysin-O which permits for greater reproducibility and for the interchange of erythrocytes of different species has been constructed.

Studies in Parahemophilia. JOHN B. FIELD and ARNOLD G. WARE, Los Angeles, Calif. (Introduced by Paul Starr).

The clinical state of plasma deficiency of accelerator globulin (Ac-Globulin, Proaccelerin, Labile Factor) may be associated with a bleeding dyscrasia. This disease has beed named parahemophilia. The number of such cases which have been studied to date are relatively few, probably numbering less than ten.

Under observation is a large, three generation family group of which nine individuals have significant reductions in plasma accelerator globulin content. Six of these individuals have levels of 50% of normal or lower. Five others who were found to have normal accelerator globulin also present a history of numerous episodes of epistaxis, easy bruising, impairment of clotting with minor cuts and severe hemorrhage with surgical procedures. Both sexes exhibit the clotting defects. The adult females also have menorrhagia and hemorrhage with parturition. At least ten other members of the family who have not been studied, have a similar history.

The most seriously ill is an eighteen year old female, the issue of two first cousins. She has had the typical subjective clotting impairment from youth with numerous severe bleeding episodes. Since the age of sixteen on, she has had several hospital admissions for long periods. She was studied in detail. Her Ac-G on numerous occasions was zero while her prothrombin concentration ranged from 120 to 140 per cent of normal. Fibrinogen was normal, prothrombin consumption was negligible. Her plasma incubated with normal plasma did not progressively reduce the average Ac-G value of the pooled sample. Given transfusions of fresh plasma, her clotting time was shortened and dental extraction was made possible. The resulting elevation of Acceleration globulin to 5% of normal was temporary and dissipated to zero within six days. Methylxanthines orally and parenterally, ACTH intravenously and vitamin K in large doses intravenously were without effect in increasing Ac-G levels.

Prolongation of Viability of the Stored Erythrocyte. CLEMENT A. FINCH * and BEVERLY W. GABRIO, Seattle, Wash.

The "storage lesion" of the erythrocyte may be defined as a composite of chemical, physical, and morphologic changes which proceed to ultimate loss of cell viability when blood is preserved *in vitro*. Because of the importance of carbohydrate metabolism in the maintenance of the erythrocyte our studies have employed the phosphate partition (adenosine triphosphate (ATP), hexosephosphates, 2,3-diphosphoglycerate, etc.) of the erythrocyte

as an indicator of the metabolic potential of the cell. Post-transfusion survival has been measured employing Cr^{51} tagging of stored erythrocytes.

Previous work from this laboratory has shown that the storage lesion is reversible to a point once stored cells are replaced in active circulation. An investigation of systems which cause such a reversal *in vitro* indicate that the ATP derivative, adenosine, is the most efficient substance in this respect. Following its addition to stored blood, there is: (1) a reconstitution of the depleted cellular organic phosphates, particularly ATP and 2,3-diphosphoglycerate; (2) associated improvements in osmotic fragility, glucose utilization, potassium: sodium ratio, and spontaneous hemolysis. Survival measurements, employing both rabbit and human erythrocytes, indicate better than 70% viability of the cells preserved in ACD + adenosine at 4° for periods of 5 to 12 weeks, while control studies show a comparable survival for only 3 to 4 weeks with blood preserved in ACD alone at the same temperature.

These studies demonstrate that membrane integrity and viability are dependent upon the maintenance of erythrocyte metabolism, and that adenosine provides important support to red cell metabolism during storage.

The Effect of Intravenous Hydrocortisone and Corticosterone on the Diurnal Rhythm in Renal Function and Electrolyte Equilibria in Normal and Addisonian Subjects. JOHN T. FINKENSTAEDT, JOSEPH F. DINGMAN, DALTON JENKINS, JOHN C. LAIDLAW and JOHN P. MERRILL,* Boston, Mass.

Nine studies on two normal subjects and two Addisonians on DOCA were performed. During control experiments, the normal subject showed diurnal rhythm in C_{In}, C_{PAH}, and adrenocortical hormone excretion. The Addisonian manifested depressed renal function without nocturnal decline. With 24-hour administration of hydrocortisone (1 mg./hr.) to the Addisonian, there was a sustained increase in renal dynamics. Slightly greater effects occurred with 10 mg./hr. The normal subject on the larger dose displayed a greater increase in renal dynamics, but diurnal variation persisted.

In control studies on normal and Addisonian subjects, electrolyte (Na, K, Cl) excretion was related to changes in electrolyte filtration rather than reabsorption. In the Addisonian, a greatly depressed electrolyte filtration produced a decreased excretion. The normal diurnal variation was absent. In the Addisonian on maintenance doses of hydrocortisone, a sustained elevation of filtration produced increased electrolyte excretion which persisted overnight. With larger doses of hydrocortisone, slightly greater increases in filtration produced an initial electrolyte diuresis. A subsequent increase in reabsorption exceeding filtration occurred. Marked NaCl retention resulted. With large doses of hydrocortisone in the normal subject, an early electrolyte and water diuresis occurred. Decreased tubular reabsorption, however, was responsible for this negative balance. As in the Addisonian, a delayed but less marked rise in reabsorption produced moderate NaCl retention. Persistent potassium diuresis was observed on large doses of hydrocortisone.

Corticosterone produced less pronounced changes in renal function and NaCl equilibria, and a more pronounced potassium diuresis.

The absence of diurnal rhythm in renal dynamics in DOCA-treated Addisonians may be due to the persistent action of this steroid or to pure 11-oxygenated corticoid deficiency. The greater effect of larger doses of hydrocortisone on NaCl reabsorption in these Addisonians may result from additional DOCA effects or to intrinsic compensatory mechanisms for salt conservation in adrenal insufficiency.

Cerebral Hemodynamics in Acute Hypotension. FRANK A. FINNERTY, JR., LLOYD WITKIN and JOSEPH F. FAZEKAS, Washington, D. C. (Introduced by Harold Jeghers).

In an attempt to establish quantitatively the critical rate of cerebral blood flow at which signs of cerebral hypoxia developed, 46 patients were studied including young and old normotensives, hypertensives, benign and malignant, and patients with spontaneous postural hypotension. Cerebral hypoxia was induced by lowering the arterial pressure acutely with intravenous hexamethonium and/or by head-up tilting.

Despite the patient's age, original level of arterial pressure, or original rate of cerebral blood flow, signs of cerebral hypoxia developed when the cerebral blood flow was reduced to 31.5 cc./min./100 gm. of brain (standard error 0.98) and the oxygen delivery to the brain fell to 5 (standard error 0.10). The level of arterial pressure at which signs of cerebral hypoxia became manifest varied between 100.7 to 40.9 mm. Hg.

The cerebral vascular resistance fell significantly in all groups, except in the subjects with spontaneous postural hypotension and malignant hypertension. In these 2 groups cerebral hypoxia developed at comparatively normal levels of arterial pressure. The cerebral vascular resistance was the highest in the malignant hypertensive group.

The cerebral metabolic rate did not change and the cerebral arterial-venous oxygen difference increased in all patients.

Angina and electrocardiographic changes were absent during cerebral hypoxia.

Our data demonstrate that there is a critical rate of cerebral blood flow and oxygen delivery to the brain at which signs of cerebral hypoxia become manifest and suggest that the brain is more sensitive to hypoxia than the heart.

Inulin and NA Para-Amino-Hippurate Clearances, Sodium and H_2O Excretion in Supine Exercising Cardiac Patients. OSCAR W. FREEMAN, JOSEPH S. WILSON and A. J. MERRILL,* Atlanta, Ga.

Other investigators have found consistently decreased Na excretion in normal and cardiac subjects during ex-

ercise, irrespective of GFR changes. Twenty four hour Na excretion studies in this laboratory, with and without exercise, cast some doubt on these findings.

The present studies, done in supine position to eliminate postural effect, include 10 cardiac subjects on digitalis and diuretics. Patients were urged to exercise consistently and strenuously for a 15 minute period. Two types of responses were noted. In 4 compensated and clinically milder cardiacs, no consistent change or correlation of GFR and Na excretion occurred. In 6 more severe cardiacs GFR consistently fell below 70 cc/minute during exercise. Without exception, GFR averaged 98.7% and Na excretion 80.7% of control in milder cardiacs, with corresponding decrease in RPF. Urine volume changes independently of GFR and Na excretion. In recovery periods, Na excretion tended to lag behind GFR in its return toward control values.

Na excretion is influenced by several factors and during *control* periods it varied irrespective of GFR. During *exercise* periods, however, when GFR fell significantly, Na excretion always showed a comparable diminution. Fifteen normals responded with no consistent change or correlation of GFR and Na excretion, as did compensated cardiacs.

These findings suggest that the results of others may have been due to posture.

Pentapyrrolidinium (M & B 2050) in the Treatment of Severe Hypertension. Edward D. Freis,* Edward A. Partenope and Lawrence S. Lilienfield, Washington, D. C.

Comparisons were made in the same, previously untreated, hospitalized patients between the ganglionic "blocking" agent pentapyrrolidinium (M & B 2050) and hexamethonium. The effective dose of pentapyrrolidinium was approximately 1/5th that of hexamethonium and the duration of hypotensive response lasted 40 per cent longer. On continuous administration there was less development of "tolerance" and less constipation than with hexamethonium. There also was little cross tolerance; patients on long term hexamethonium treatment, who had become resistant to the hypotensive effects of that drug requiring increased dosages, responded to doses of M & B 2050 which were as little as 1/10th to 1/20th the previously required amount of hexamethonium.

Although oral absorption of M & B 2050 was poor similar to hexamethonium the hypotensive response following oral administration was more uniform and predictable. Constipation was less severe and when present usually could be controlled with oral neostigmine. Ileus was not seen. The other side effects of ganglionic blockade were similar to those observed with hexamethonium. Twenty-seven patients have been treated for periods of 5 to 8 months on M & B 2050 alone. Twenty-two of these have been controlled without severe reactions although dosage adjustment was critical. A few have required parenteral therapy.

In another group of patients treated with M & B 2050 the addition of reserpine produced further hypotensive effects in approximately half. 1-Hydrazinophthalazine was found to produce additive effects in a similar percentage. Thirty-two patients have been treated for 5 to 8 months with combinations of oral M & B 2050, reserpine and 1-hydrazinophthalazine. Withdrawal of the latter 2 agents resulted in some increase of average blood pressures in approximately half of the patients.

It is concluded that M & B 2050, although still producing many of the side effects of ganglionic "blockade" has the following advantages over hexamethonium: (1) smaller dosage requirement, (2) longer duration of hypotensive effect permitting less frequent administration, (3) more predictable response to oral therapy, (4) less gastrointestinal atony and (5) less development of "tolerance."

A Study of the Blood Volume in Congestive Heart Failure. Robert K. Funkhouser and Walter H. Pritchard,* Cleveland, O.

Recent studies of cardiac failure using labelled red cells have failed to demonstrate a significant elevation in blood volume, a result which is at variance with the findings of previous investigators using labelled albumin. This study was undertaken to verify both of these findings and if possible to interpret their implications.

Twenty hospitalized patients were selected; eleven controls and nine patients with varying degrees of cardiac edema. In each patient, red cell and blood volumes were estimated by the Cr^{51}-labelled erythrocyte method of Gray and Sterling. Venous samples were drawn over a period of one hour, and immediately thereafter, an injection of I^{131} human serum albumin was given in order to estimate plasma and blood volumes. Hematocrit of each blood sample assayed for radioactivity was determined.

Statistical analysis of the relationship of red cell, plasma, and total blood volume to height, "wet" weight, and surface area was carried out for erythrocyte and albumin dilutions. Results for weight and surface area showed qualitatively similar but quantitatively different relationships to each measured blood volume. The cardiacs demonstrated the following statistically significant differences from the control group:

(1) By albumin dilution, the plasma and blood volumes were much greater.

(2) By erythrocyte dilution, red cell masses were elevated; total blood volume was also elevated even when differences in physical dimensions were considered, but to a lesser extent than by albumin dilution.

This discrepancy in the extent of the difference can be explained on the basis of either of two hypotheses advanced in the past by others: (1) an altered distribution of red cells in the circulation in congestive failure, or (2) the presence in congestive failure of a larger than normal, rapidly equilibrating, extravascular space of albumin distribution.

The Influence of Androgen and Estrogen on the Association of Lipids with Serum Globulins; Ultracentrifugal and Electrophoretic Studies of Problems in Atherosclerosis. ROBERT H. FURMAN, R. PALMER HOWARD and LOYAL L. CONRAD, Oklahoma City, Okla. (Introduced by Robert H. Bayley).

This study was supported in part by research grants from the National Heart Institute (H-1429) and the Smith, Kline and French Research Foundation.

Estrogens have tended to become associated with prophylaxis against, or delay in the genesis of atherosclerosis, both in chicks (Katz et al.) and human (Barr et al.). The latter note the greater proportion of cholesterol extractable from serum alpha globulin fractions in the female and suggest that this alpha lipoprotein-sex relationship may be the factor responsible for the relative immunity to coronary atherosclerosis enjoyed by premenopausal women. Lewis, however, has demonstrated that alpha-1 lipoproteins, while more concentrated in women than men, continue to increase in concentration beyond the menopause, a time when the female's "immunity" is lost.

Androgen, especially methyl testosterone, and estrogen, chiefly conjugated equine estrogens were studied in relation to their effects on serum lipids and lipoproteins (the latter by means of the analytical ultracentrifuge utilizing a solvent density of 1.21 as described by Lewis) and the electrophoretic distribution of serum proteins.

A variety of human subjects including eunuchoidal males, postmenopausal females, and patients with pituitary dysfunction were studied. Hypogonadism in either sex is associated with increased alpha-1 lipoprotein. Androgens (especially methyl testosterone) are consistently effective in reducing or eliminating alpha-1 lipoprotein. Premarin failed to block this effect. Alpha-2 and beta lipoproteins responded less uninformly. Alpha-2 lipoprotein tended to decrease and to become ultracentrifugally distinct from beta lipoprotein as alpha-1 lipoprotein increased. Diethyl stilbestrol markedly increased alpha-1 lipoprotein in the normally endrogenic male. Serum cholesterol and phospholipid relationships during steroid therapy failed to reveal changes suggesting higher phospholipid concentrations in alpha-1 lipoproteins.

Conventional electrophoresis showed no steroid-induced changes indicative of the marked lipoprotein changes noted with the ultracentrifuge.

It is concluded that the gonadal steroids alter serum lipoproteins by influencing the amount and/or nature of lipids associating with globulins. The hypogonadal state appears to be associated with higher alpha-1 lipoprotein concentrations in both sexes.

Respiratory and Renal Adjustments to Alterations in Acid-Base Balance Induced by Diamox, 2-Acetylamino-1,3,4-Thiadiazole-5-Sulfonamide, in Normals and in Patients with Chronic Lung Disease. MORTON GALDSTON,* New York, N. Y.

Recent studies have demonstrated a linear relationship between renal tubular reabsorptive capacity for bicarbonate bound base ($H - Na$ transfer) facilitated by carbonic anhydrase and acutely induced respiratory variations in arterial pCO_2 in the regulation of acid-base balance. It seemed important to compare the respiratory system of individuals without heart, lung or kidney disease with individuals with advanced pulmonary emphysema and elevated arterial pCO_2 with regard to sensitivity to depletion of serum bicarbonate (metabolic acidosis). This was brought about by ingestion of Diamox, a potent renal carbonic anhydrase inhibitor, in doses of 2.2 to 6.3 mgm/kg. every 6 hours for 1 to 3 months.

Though members of both groups of patients often exhibited approximately the same degree of Diamox induced decrease in serum $BHCO_3$ (6.0 to 8.0 mEq/L), their respiratory response to the resulting calculated fall in arterial pH (7.18–7.26) varied considerably. Normals consistently responded with more effective alveolar ventilation, resulting in decreased arterial pCO_2. This caused further inhibition of renal tubular reabsorption of bicarbonate bound base to decrease serum $BHCO_3$ an additional 2 mEq/L. As a result, these individuals were able to maintain a slightly lowered arterial pH of 7.30 to 7.35. In contrast, the abnormals exhibited either no respiratory response or one which resulted in at most an additional fall of 1 mEq/L serum $BHCO_3$. Their respiratory acidosis therefore became more intense and occasionally was complicated by metabolic acidosis. The potential handicap of such bicarbonate bound base depletion without adequate respiratory compensation is of considerable clinical importance where further carbon dioxide retention and/or serum $BHCO_3$ depletion is likely to occur.

Diamox did not induce any specific alterations in minute volume of respiration and gas exchange. Diuresis was evident only during the first few days of administration.

The Study of Human Serum Phosphatide Patterns in Normal and in Pathologic States as Studied by Quantitative Paper Chromatographic Methods. MENARD M. GERTLER and JACOB KREAM, New York, N. Y. (Introduced by Bernard S. Oppenheimer).

Much attention has been given in the past few years to the role of serum phosphatides in atherosclerogenesis. Since this lipid fraction is heterogeneous it would be desirable and of interest to distinguish the differential changes, if any, in the individual phosphatide components. Quantitative paper chromatographic methods offered interesting possibilities in that they could be adapted, both to the quantitative and qualitative microanalysis of serum phosphatide components such as choline, serine and ethanolamine containing types. Such methods have been developed in our laboratory during the past year permitting direct phosphatide analysis on small samples of serum. Basically the method consists of (a) extraction of the phosphatide components from serum, (b) hydrolysis of the phosphatide extracts, and (c) separation of the nitrogenous components (choline, serine and ethanolamine) of

the resulting hydrolysate by means of paper partition chromatography and their quantitative estimation by means of quantitative colorimetry.

Data will be presented from (a) 22 normal males of various ages, (b) 32 individuals who have experienced myocardial infarction, (c) 5 pregnant women at various stages of gestation, and (d) 25 samples of serum obtained from cord blood. In addition, data will be presented showing the differential changes observed in the total serum phosphatides and the serum phosphatide components during the administration of various oestrogenic materials to (a) males with coronary heart disease and (b) males with carcinoma of the prostate.

Much information concerning the serum phosphatide pattern has already been accumulated as a result of this investigation. Phosphatides containing choline (e.g. lecithin and sphingomyelin) invariably appear to be the predominant type (60–100 per cent) found in human blood serum. Practically all the samples analyzed, however, do contain appreciable amounts of phosphatidyl ethanolamine and phosphatidyl serine indicting that the so-called cephalin fraction is heterogeneous. The ratio of phosphatidyl serine to phosphatidyl ethanolamine is variable. In certain samples serine appears to predominate over ethanolamine while in others this situation is reversed.

Coagulation Defect and Hemorrhagic Diathesis Due to an Acquired Inhibitor of Antihemophilic Factor. ROBERT GOLDSTEIN, Boston, Mass. (Introduced by Samuel L. Gargill).

A circulating inhibitor of antihemophilic globulin has been disclosed as the cause of a recently developed bleeding diathesis in a 40 year old male with a negative family and past history. The disorder was characterized by easy bruising, spontaneous hematuria, hematomata, and hemarthroses. Extensive routine clinical and laboratory investigation revealed no other abnormalities or diseases including disseminated lupus.

Coagulation studies revealed: normal bleeding time; prolonged clotting time (30–120 minutes); extremely retarded prothrombin consumption; normal platelets; normal prothrombin time; normal plasma prothrombin, Ac-globulin, pro-Spca, fibrinogen, and anti-thrombin activity; questionably significant increase in plasma anti-thromboplastin (human brain). The defective prothrombin conversion was as readily rectified by small amounts of thromboplastin as that of a known hemophiliac, but not by fresh normal blood or plasma. Moreover, as little as one part of the patient's plasma to nine parts of freshly shed normal blood caused retarded consumption in the mixture. By utilizing the prothrombin consumption test to determine the antihemophilic, PTC and PTA activity of plasma and plasma mixtures, the site of action of the anticoagulant could be delineated. The patient's plasma did not correct the retarded prothrombin consumption in hemophilia, but could correct that of PTC and PTA deficiency. Admixture of patient's plasma or serum with normal plasma markedly reduced the antihemophilic activity of normal plasma but left its PTC and PTA activities intact, thus indicating the specific inhibition of the antihemophilic factor. This inhibitor was not adsorbable by $BaSO_4$ and survived heating at 56° C. for 30 minutes. Striking similarities between the clotting abnormality of this subject and that seen in resistant hemophiliacs were noted.

The etiology remains obscure. There had been no blood or plasma transfusions before onset of illness. A history of alcoholism and exposure to naphtha and gasoline suggest the possibility of a toxic reaction. Cortisone therapy was ineffective.

The Role of the Adrenals in the Response to Endotoxin. ROBERT A. GOOD and LEWIS THOMAS,* Minneapolis, Minn.

Increased vulnerability to the lethal effect of gram negative bacterial endotoxins occurs in adrenalectomized animals, and this has been interpreted to indicate that resistance to endotoxin involves adrenal function.

The manifestations of intoxication by endotoxins in normal animals include fever, leucopenia, prostration, and the local and generalized Shwartzman reactions. When repeated injections of small doses are given, a state of refractoriness develops, during which none of these responses can be elicited.

It is known that the enhanced susceptibility of adrenalectomized animals is prevented by cortisone. Moreover, cortisone has been found to protect normal adult rabbits against the prostrating and lethal effects of meningococcal endotoxin. On the other hand, no protection against the local or generalized Shwartzman reactions occurs; instead, the reactions are more readily produced in cortisone-treated animals. Furthermore, daily injections of cortisone prevent the development of the refractory state.

In view of the paradoxical differences between the effects of cortisone on different toxic manifestations, the responses to endotoxin in adrenalectomized rabbits were studied.

Typical local Shwartzman reactions occurred in each of 12 adrenalectomized rabbits. The generalized reaction, with renal cortical necrosis, was produced in 3.

Normal rabbits were given repeated injections of meningococcal endotoxin until they were completely refractory to fever, leucopenia, prostration, and the Shwartzman reaction. At this time both adrenals were removed. Four days later, the degree of resistance was found to be unchanged. The refractory state was also produced in rabbits which had been adrenalectomized before any exposure to endotoxin.

It is concluded that 1) although the adrenals may be implicated in some of the reactions to endotoxin, they are not directly involved in the Shwartzman reaction, and 2) the development of refractoriness to endotoxin is not a function of the adrenals.

Tubular Reabsorption of Protein in Experimental Nephritis. HOWARD GOODMAN and JAMES BAXTER,* Bethesda, Md.

With acceptance of the concept of a normal glomerular filtration and tubular reabsorption of protein, the question arises whether there is an increase or a decrease in the tubular reabsorption of protein in instances of proteinuria. The dye T-1824 has been widely used as a tag for serum proteins, and specifically in studies of the renal clearance of albumin (Chinard, Lauson and Eder, J.C.I. **31**, 895, 1952). In addition, the accumulation of the blue dye in droplets in the proximal convoluted tubule cells has been described as a measure of the tubular reabsorption of protein in normal and renin-injected rats (Sellers, Smith, Narmorston and Goodman, J. Exp. Med. **96**, 643, 1952). In the present experiments, 2.5 mgm. of T-1824 is injected intravenously into normal rats and rats with proteinuria due to anti-kidney serum nephritis and uranium nephritis. With tubular injury due to the administration of uranium, there is less than normal accumulation of the blue dye in the renal tubules. In rats with the nephrotic syndrome produced by nephrotoxic serum, the cortical kidney tubules appear intensely blue due to the accumulation of dye droplets in the tubule cells.

These results suggest that tubular reabsorption of protein is impaired in rats with proteinuria due to uranium nephritis. In proteinuria due to the administration of nephrotoxic serum however, the tubules are reabsorbing greater than normal amounts of protein, and in this instance the proteinuria would seem to be due solely to an increase in glomerular permeability to protein.

Observations on the Hyponatremia Following Mitral Valvulotomy. ALLAN V. N. GOODYER* and WILLIAM W. L. GLENN, New Haven, Conn.

Twenty-seven patients subjected to mitral valvulotomy were studied before and after operation with regard to the incidence, extent, and mechanism of post-operative hyponatremia. Balances of water, sodium, and chloride were carried out on seventeen patients. The serum sodium fell below 131 meq./l. in thirteen cases in the immediate post-operative period, the serum chloride fell below 99 meq./l. in all cases but one and below 90 meq./l. in thirteen cases. The small negative balances of sodium and chloride were inadequate, by themselves, to account for the observed hyponatremia in all but three cases. Intracellular shifts of sodium (using the chloride space as a reference) were appreciable (over 100 meq.) in only five patients.

The retention of water (allowing for insensible losses) was enough to explain the observed hyponatremia in all but three cases, although the level of fluid *intake* was not well correlated with the occurrence of hyponatremia.

These results indicate the presence of an unusually prolonged antidiuretic stimulus in these patients, the etiology of which remains uncertain. Except in a few cases it is unnecessary to correct the hyponatremia with hypertonic salt, since it is usually a transient, limited, and essentially asymptomatic phenomenon.

Quantitative Studies of the Circulation in Aortic Valvular Disease. RICHARD GORLIN, IAN K. R. MCMILLAN, MICHAEL B. MATTHEWS, RAYMOND DALEY and W. E. MEDD, Boston, Mass. (Introduced by Eugene C. Eppinger).

No data are available concerning left ventricular (LV) systolic pressure, regurgitation volume, LV stroke work or pressure-flow relationships in aortic valvular disease because the first two values, necessary in calculation, cannot be measured directly.

In 1952, McMillan described a cyclic pump wherein aortic valves could be perfused with water under simulated in vivo conditions and motion pictures taken of the functioning valve. The hearts of five patients were so studied at post mortem. From the films stenotic and regurgitant valve areas were measured planimetrically. Utilizing orifice formulae relating orifice area, pressure and flow, with data obtained at prior cardiac catheterization, regurgitant volume and LV systolic pressure were estimated. Total cardiac output (systemic plus regurgitant) and LV stroke work were calculated.

In pure stenosis (0.4 $cm.^2$ area; normal 4 $cm.^2$) LV systolic pressure and work were twice normal. In similar stenosis (0.4 $cm.^2$) with insufficiency (0.3 $cm.^2$ area), regurgitant volume was one-half systemic, LV systolic pressure twice and work thrice normal. In similar stenosis (0.4 $cm.^2$) with tight mitral stenosis, however, because of the very low cardiac output, LV systolic pressure was only slightly increased and work was below normal. In less severe stenosis (0.7 $cm.^2$) with insufficiency (0.4 $cm.^2$) regurgitant volume equalled systemic, LV systolic pressure was two and work four times normal.

Remarkably constant LV systolic pressures between 200–250 mm. Hg were found at rest and on effort. Accordingly, aortic valve gradient and flow had an upper limit, thus fixing stroke output for each patient. This appeared related to the orifice phenomenon; as orifice decreased or flow increased (regurgitation) the exponential pressure-flow curve became flat so that further increases in pressure effected virtually no increases in flow. Critical orifice area for pure stenosis was 0.5 $cm.^2$, but was correspondingly larger if there was associated insufficiency.

Brachial Arterial Pulse Wave Contours in Valvular Heart Disease. DAVID G. GREENE and CHARLES E. MCLEAN, Buffalo, N. Y. (Introduced by John H. Talbott).

Photokymographic records of intra-arterial pulse pressures have been made at a paper speed of 120 mm per second with a high frequency blood pressure recording system. Analysis of the resultant form shows several variations among the normal subjects studied, including smoothly rounded peaks of pressure during systole, double

peaks, and notches or slurs on both the anacrotic and catacrotic limbs. Although the ascent from the diastolic level to the peak of systole is usually a smooth continuous curve with no sudden changes in slope, in three out of 26 normal subjects there was an anacrotic notch. Such a notch was seen more frequently in patients with various lesions of the cardiovascular system. Two out of four patients with pure aortic stenosis demonstrated such a notch. Six out of nineteen patients with mitral valvular disease and clinically normal aortic valves also showed an anacrotic notch. It was seen in all seven patients studied who had aortic stenosis combined with mitral valvular disease. It was noted in two of seven patients with aortic insufficiency. In the normal subjects the anacrotic notch appeared more than 70 per cent of the way up the ascending limb of systole, and the same high position of the anacrotic shoulder was noted in the patients with mitral disease. Patients with pure aortic stenosis, or with aortic stenosis combined with mitral valvular disease, had a lower position of the anacrotic notch. Three of the seven patients with aortic insufficiency showed obliteration of the dicrotic notch, so that the catacrotic limb was a smooth unbroken fall.

The Effect of Chelating Agents Upon the Metabolism of Stable and Radio-Metals. J. GREENBERG, H. E. HART, R. LEWIN, H. SPENCER, K. G. STERN and D. LASZLO, New York, N. Y. (Introduced by Louis Leiter).

Chelating agents present a new tool to study deposition and removal of physiologic and foreign metals, stable and radioactive, in man. This report deals with an analysis of the underlying mechanism of these processes, selecting as models two radioactive metals which are known bone seekers. Following the intravenous injection of La^{140} and of Y^{90} chloride high and sustained plasma levels, high uptake by liver and low urinary excretion were observed. It is assumed that these metals, upon being injected into the blood stream, formed high molecular complexes which were not excreted by the kidneys but were transferred to, and mainly deposited in, the liver. After intravenous injection of La^{140} ethylenediaminetetraacetic acid, (EDTA), Y^{90} nitrilotriacetic acid (NTA), Y^{90} EDTA and Y^{90} EDTA in the presence of excess Ca-EDTA, the cumulative urinary excretion ranged from $<5\%$ to $>85\%$ respectively. These excretory differences are in agreement with the differences in the binding strengths of these metal chelates. Yttrium injected as EDTA-chelate rapidly disappeared from the blood stream, diffused into the tissues, re-entered the vascular compartment and large amounts were promptly excreted in the urine. Yttrium injected as the NTA-chelate also rapidly disappeared from the blood stream. There was no evidence of re-entry, and there was fixation by various tissues, with areas of concentration in bone, liver, spleen and kidneys. The urinary excretion was minimal. These differences in the metabolism of metal chelates may provide a method to measure the ability of biological systems to dissociate metal chelates.

Remission in Nephrotic Syndrome in Children Receiving ACTH. LAWRENCE GREENMAN and T. S. DANOWSKI,* Pittsburgh, Pa.

In attempts to influence underlying disease rather than just deliver edema 30 children with nephrotic syndrome of 22 months duration or less were treated with ACTH, 100 mgm daily for 28 days, alone or with nitrogen mustard, while on penicillin and diets containing 10 milliequivalents of sodium and 150 milliequivalents potassium.

In 15 of 19 patients edema was delivered prior to ACTH therapy following dextran ("Plavolex"), polyvinylpyrrolidone, urea or a sodium-free diet. Of the patients in whom edema was present at the time the regimen outlined above was started 11 delivered the excess fluid within two weeks, one at the end of four weeks and two after cessation of ACTH; three required paracentesis.

Though decreases in albuminuria and restoration of serum proteins and cholesterol toward normal began during treatment, maximum improvement was often not recorded until several weeks following treatment. Twenty-eight of the 30 children have been followed for 1 to 26 months: 64% were normal and 28% greatly improved at the last visit. Relapses, responding to re-treatment, occurred in 21% after remission lasting 3 to 24 months. There is no evidence that nitrogen mustard improved the immediate or the end results.

Three patients developed hyponatremia; in two this followed paracentesis and reaccumulation of ascites. Two developed convulsions with hypertension and serum sodium concentrations of 112 and 116 milliequivalents per liter; one was left with cerebral atrophy following respiratory failure and anoxia. Temporary mental depression occurred in another patient. Mild upper respiratory infections occurred frequently; one patient developed peritonitis.

At this point the results suggest that the regimen of ACTH-low-sodium-high potassium-penicillin produces a high percentage of remissions in the nephrotic syndrome of children, but that recurrences which respond to re-treatment are relatively frequent. The marked sodium restriction may result in sodium deficits and convulsions.

The Location of the Hypothalamic Area Controlling the Secretion of Thyrotropin by the Pituitary. MONTE A. GREER and HOWARD ERWIN, Bethesda, Md. (Introduced by Harry Eagle).

Bilateral hypothalamic lesions from anterior commissure to mammillary bodies were produced in 424 young adult female rats. Lesions were 0–4 mm. above the base of the skull and 0–1.5 mm. lateral to the midline. After allowing 1–2 weeks for post-operative recovery, the animals were fed 0.15% propylthiouracil for 10–14 days. Average thyroid weight in 18 similarly fed normal controls was increased from 7–8 mg./100 gm. body wt. to 16.3 ± 1.2 mg.; thyroid cell height was also greatly increased. Of the operated rats, 364 had a normal hypertrophic thyroid response and 8 had only slight thyroid hypertrophy. In 54, thyroid weight was only 4–8.5 mg. and there was no increase in thyroid cell height. In the

last group, the lesions had either destroyed the paraventricular nuclei or were in the midline between the paraventricular nuclei and the infundibulum. In the latter group there was marked cellular loss or almost total disappearance of the paraventricular nuclei, even though the paraventricular area was not directly damaged. This area was not affected in the 364 animals with normal thyroid response.

Seven of the 8 animals with slight thyroid hypertrophy had lesions which partially but not completely destroyed this region. Supraoptic nuclei were usually normal and their complete destruction did not prevent thyroid hypertrophy. Similarly, lesions directly above, rostral, or lateral to the paraventricular nuclei or its caudally extending path to the pituitary did not interfere with thyroid hypertrophy. These findings strongly indicate that the region of the paraventricular nuclei is intimately concerned with the elaboration of thyrotropin by the pituitary.

Body Fluids and Electrolyte Balance in Myxedema. S. I. Griboff, R. Wiener, J. Eisenberg and A. Iannaccone, New York, N. Y. (Introduced by L. J. Soffer).

Body fluid distribution was studied in one patient with primary, and one with pituitary myxedema. Both subjects had normal extracellular fluid (thiosulfate) and total body water (antipyrine) spaces before treatment. During the period of thyroid medication both showed temporary expansion of thiosulfate space with subsequent fall toward control levels, while the antipyrine space remained essentially unaltered. Both patients showed weight loss and increased urine volume. The subject with primary myxedema showed negative sodium and chloride balances (previously positive), continued positive potassium balance, and elevated serum mucoprotein and hexosamine during the period of therapy, suggesting breakdown of extracellular myxedematous tissue. Expansion of thiosulfate space in both subjects, without comparable change in total body water, might signify redistribution of body fluid but more probably represents increased extracellular penetrability of thiosulfate ion, with consequent increase in measurable extracellular fluid space.

It was previously found that in normal individuals cortisone causes transient increase in extracellular fluid space without change in body weight. Before thyroid therapy the patient with primary myxedema received cortisone for 14 days (150 mg./day), which produced no significant change in body fluid compartments. After 54 days of thyroid medication the addition of cortisone for 17 days produced increased expansion of thiosulfate space without altering total body water or weight. Sodium and chloride balance remained negative, potassium balance positive, and serum mucoprotein and hexosamine elevated. These findings suggest intensification of thyroid effect following cortisone administration, with further breakdown of myxedematous tissue, and indicate that thyroid hormone is necessary for production of cortisone-induced increase in thiosulfate space.

Studies on the Stimulating and Depressant Effects of Acetylcholine on Neuromuscular Function in Man. David Grob,* Richard J. Johns, Victor A. McKusick and A. McGehee Harvey,* Baltimore, Md.

The response to the intra-arterial injection of acetylcholine (ACh), the chemical mediator of neuromuscular transmission, was studied in 26 normal subjects by recording the stimulating and depressant effects of this compound. Injection of ACh produced prompt stimulation of muscle fibers, attributable to depolarization of motor end plates, resulting in a visible twitch . This was followed by depression of neuromuscular transmission, evidenced by a decrease in the muscle action potentials in response to maximal motor nerve stimuli and by weakness. The depressant effect of ACh, which was proportional to the logarithm of the amount injected, is attributable to persistent depolarization of motor end plates, rendering them temporarily incapable of activation.

Precise measurement of these effects of ACh provides a tool for studying the physiology and pharmacology of neuromuscular function, and its alterations in disease. The influence of various types of neuromuscular block on the effects of ACh was studied. Following injection of d-tubocurarine there was elevation in the threshold to stimulation and depression by ACh. d-Tubocurarine block was reversible by ACh. Following block by decamethonium the effects of ACh were not altered, and the block was not changed by ACh. Following anticholinesterase compounds, such as neostigmine or edrophonium, the effects of ACh were greatly enhanced and prolonged. The degree of block increased with successive nerve stimuli, probably because of accumulation of depressant concentrations of ACh.

Increased reactivity to ACh and prolongation of its effects were also observed after upper motor neurone lesions. These changes were not due to decreased cholinesterase activity. The most striking increase in reactivity (20 fold) occurred in a patient who had recently developed basilar artery thrombosis. Since this patient also had myasthenia gravis, the increased reactivity is of interest in view of the suggestion by some that myasthenia gravis is due to decreased responsiveness to ACh.

Nucleic Acid Metabolism in Chronic Lymphatic Leukemia. Leonard Hamilton, New York, N. Y. (Introduced by Joseph H. Burchenal).

Adenine -8-C^{14} (200 microcurie, 270 mg.) was given intravenously to a patient (age 46) with chronic lymphatic leukemia. Thirty-three collections of lymphocytes have been made at intervals over a period of 300 days. The ribose nucleic acid (RNA) and desoxyribose nucleic acid (DNA) have been separated from the cells. The individual purines from each were separated and their radioactivities determined.

It was found that adenine was incorporated as such into the RNA, and to a smaller extent into the DNA, and that there was an eventual extensive transformation of the adenine into RNA and DNA guanine. Radioactivity

of RNA adenine appeared to reach a maximum at day 5 (560 counts/min./μM.) and in RNA guanine at day 14 (180 counts/min./μM.); activity in DNA adenine reached a maximum between days 7 to 11 (100 counts/min./μM.). The DNA guanine reached an activity (70 counts/min./μM) equal to that of the DNA adenine on about the 30th day. The activity of RNA adenine and guanine likewise reached approximate equality at 30 days and the activities of the purines in each nucleic acid decreased in parallel thereafter.

The decline in activity in both RNA and DNA could be divided into two phases—an initial more rapid component of approximately 2 months duration and a residual slow component showing a half-time of the order of 300 days. The decline in activity of the RNA greatly exceeded that of the DNA during the initial period, but during the latter period the declines in activities of the RNA and the DNA were more nearly parallel. The prolonged retention of the isotope incorporated into the purines was such that at approximately 300 days, activity in the DNA was still more than one-third of the peak activity, and was greater than that in the RNA.

These results suggest that: (a) leukemic lymphocytes survive for very much longer periods than have hitherto been suspected, namely apparently for over a year, or (b) that leukemic lymphocytes have a remarkable capacity to re-utilize specifically, and with great efficiency, large fragments of the nucleic acids or nucleoproteins of their progenitors.

The implications of these observations will be discussed with reference to the utilization of various precursors by lymphocytes and other cell types.

Capacity of Seriously Wounded Patients to Produce Antibody. W. Paul Havens, Jr.,* Don G. Bock and Irwin Siegel, Philadelphia, Pa.

The capacity for production of antibody by young soldiers with severe inanition and hypoproteinemia associated with poor healing of wounds or neurologic injuries was investigated as one aspect of the metabolism of proteins in such patients. Fifteen Schick-negative patients and eleven Schick-negative healthy controls were inoculated with 50 Lf purified diphtheria toxoid, and the amounts of circulating antitoxin were determined at frequent intervals during the following month. The serum albumin before inoculation ranged from 2.0–4.4 gm.%, averaging 3.3 gm.%, in the patients, while the amounts were within normal limits, averaging 4.3 gm.%, in the controls. There were no significant qualitative or quantitative differences in the patterns of immunologic response in the two groups. The maximum measured amounts of antitoxin produced by the patients averaged 38.85 units/ml. serum, while the controls averaged 26.50 units/ml. serum. No apparent relationship existed in either group between the amounts of serum albumin or total proteins present immediately before inoculation with diphtheria toxoid and the amount of circulating antitoxin which subsequently developed. There was also no evident relationship between the capacity to produce antibody and the age of the patient, clinical condition, degree of malnutrition, or resistance to infection. The only significant relationship determined in both groups was between the amounts of circulating antitoxin found before the Schick test and the maximum absolute increases in antitoxin attained after inoculation of toxoid. In general, the greater the amount of pre-Schick circulating antitoxin, the greater was the maximum measured amount subsequently attained.

Cardiocirculatory Performance in Atrial Flutter. Réjane M. Harvey, André Cournand and M. Irené Ferrer,* New York, N. Y.

A series of nine patients with atrial flutter have been studied using the cardiac catheterization technique and measuring cardiac output (Fick principle), blood volume, right ventricular, pulmonary arterial and peripheral arterial pressures. The basic pressure curves illustrate the constant occurrence of a mechanical auricular activity with each electrical flutter wave. Right ventricular and pulmonary artery curves as well as the atrial curve itself demonstrate the impact of each of these rapid atrial systoles. This is in sharp contrast to the pressure curves in atrial fibrillation where individual atrial systoles cannot always be identified. Furthermore the pressure pattern of tricuspid insufficiency, so commonly observed in atrial fibrillation, rarely occurs in atrial flutter.

A patient without evident heart disease in atrial flutter has been compared to the group with the arrhythmia and a variety of intrinsic heart diseases. In this latter group four patients in congestive failure were acutely digitalized, and two of these were studied again after conversion to normal sinus rhythm. Finally two patients in flutter but not in congestive failure were observed at rest and during exercise and then under these two conditions after conversion to sinus rhythm. From the accumulated data it is evident that this arrhythmia is associated with a low cardiac output only in those subjects with intrinsic heart disease, since the blood flow was normal in the one man without disease. In addition, the resting cardiac output rose after conversion to sinus rhythm in two subjects, which is reminiscent of observations in atrial fibrillation made by other observers. In general, a small pulmonary artery pulse pressure and a low level of pulmonary hypertension were found in those subjects with atrial flutter and congestive failure in contrast to patients in failure with sinus mechanism.

The Fate of Hydrocortisone-4-C^{14} in Man. Leon Hellman, H. Leon Bradlow, Jerome Adesman, David K. Fukushima, J. Laurence Kulp and T. F. Gallagher, New York, N. Y. (Introduced by C. P. Rhoads).

Hydrocortisone-4-C^{14} was given intravenously to a series of human subjects in both tracer and therapeutic quantities and the following observations were made concerning the metabolism of this principal adrenal hormone.

1. The routes, rate, and amount of excretion have been defined. 70–80% of the administered radioactivity was

eliminated in urine within 24 hours at a rate characterized by a half-life of 3.6 hours. An additional 6% appeared in urine during the next two days and 9% was recovered in feces in four days.

2. The absence of hormone breakdown, as indicated by loss of C^{14} from ring A, was established by the failure to find $C^{14}O_2$ in expired air by the most sensitive method of radiocarbon analysis.

3. The metabolism of the hormone was independent of the amount administered since the rate and cumulative excretion were unchanged with amounts of hydrocortisone from 0.25 to 100 mg.

4. The original hydrocortisone was rapidly subjected to chemical alteration since 85% of the material excreted in the urine was in a conjugated form within two hours. The concentration of the free hormone and its metabolites in the circulation was measured and, as demonstrated by isotopic dilution, a minimum of 87% of the circulating radioactivity was present in a form other than the original hydrocortisone within fifteen minutes after infusion.

5. By these criteria, the metabolism of hydrocortisone was shown to be independent of physiologic and pathologic variations induced by disease.

6. The problem of "tissue demand" for hormone, as evidenced by alterations in these processes, was explored in an adrenalectomized oophorectomized patient and no differences were observed in the fate of tracer doses given at a time when all replacement thrapy was withdrawn and again when the tissues of the same subject were saturated with hormone.

Some Metabolic Effects of Glutathione Given Intravenously in Man. DOROTHY H. HENNEMAN, Boston, Mass. (Introduced by Mark D. Altschule).

Large doses of glutathione (Schwarz "Triptide") were injected intravenously in three normal men and thirty-one psychotic patients in a total of forty-nine experiments. No toxic reactions occurred if certain precautions were observed.

In the fasting state giving glutathione decreases the total serum inorganic phosphate, whole blood lactic and pyruvic acids, plasma citric acid, and alpha-keto-glutaric acid.

Four and twelve gram doses administered during thirteen oral or intravenous glucose tolerance tests had no effect on the normal glucose tolerance. However, larger doses, i.e. 0.5 gm/kg., increased the normal tolerance of one normal male and changed the abnormal carbohydrate metabolism of one psychotic patient to normal. In four psychotic patients a dose of four grams significantly decreased the lactic acid rise observed after administration of glucose; no change in glucose tolerance occurred with this small dose.

Glutathione in doses of 12 gm. and of 0.5 gm/kg. had no effect on the carbohydrate changes induced in the oral or intravenous glucose tolerance by a one to three day course of Compound F, or ACTH, in five psychotic patients and one normal subject.

A dose of 0.5 gm/kg. of glutathione increased the reaction to insulin in one psychotic patient. The abnormal carbohydrate response to epinephrine became normal in one psychotic patient after the administration of glutathione.

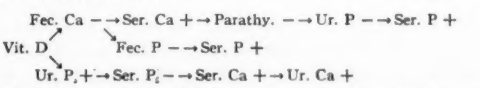
Depending upon the dose administered, glutathione increased the fall in total serum inorganic phosphate due to glucose, levulose, pure sodium d-lactate, insulin, epinephrine, and the combined action of ACTH and glucose.

The Mechanism Responsible for Hypercalcuria in Sarcoid. PHILIP H. HENNEMAN, EVELYN L. CARROLL and ELEANOR F. DEMPSEY, Boston, Mass. (Introduced by Fuller Albright).

A male patient with sarcoid was investigated with a complete balance study for calcium, phosphorus, nitrogen, sodium, potassium, and chloride. The study was divided into 6 days fore-control, 30 days oral cortisone acetate therapy, and 12 days after-control.

Initially the patient exhibited hypercalcemia and hyperphosphatemia and markedly negative calcium and phosphorus balances. On a diet containing 292 mg. of calcium, the fecal calcium was only 23 mg. while the urinary calcium averaged 930 mg. per day. Cortisone produced a fall in the serum calcium, a steplike fall in urinary calcium to a minimum of 216 mg, and a rise in fecal calcium to a maximum of 417 mg. On discontinuing cortisone the urinary calcium had risen to 448 mg. per day by the 12th day.

The mechanism responsible for hypercalcemia and hypercalcuria in this patient appears, at least partly, to be an excessive absorption of calcium from the gastrointestinal tract in a pattern suggesting hypervitaminosis D. That excessive absorption is not the entire explanation is suggested by a urinary calcium excretion markedly in excess of dietary intake, a relation which could be explained, however, by the second or parathyroid-hormone-like action of vitamin D. These two actions of vitamin D are considered to be:



Note is made of the finding of unusually low fecal values for nitrogen, sodium, and potassium, a finding noted previously during overdosage with dihydrotachysterol.

In confirmation of this study data are presented from balance studies on two other patients with sarcoid. In each instance the finding of a very low fecal calcium coupled with a very high urinary calcium supports the suggestion that the mechanism responsible for hypercalcuria in sarcoid may be excessive endogenous production of vitamin D-like-substances.

Evidence of a Regulatory Role of the Thyroid in the Excretion of Nitrogen During Stressful Life Experiences in Man. BASIL S. HETZEL, WILLIAM W. SCHOTTSTAEDT and WILLIAM J. GRACE, New York, N. Y. (Introduced by Harold G. Wolff).

Serum protein bound iodine (P.B.I.) and urinary excretion of nitrogen, sodium, and potassium before, during, and after experimentally induced stressful life experiences

have been observed in euthyroid and in hypothyroid subjects maintained on dessicated thyroid.

In 14 studies on 13 euthyroid subjects, mean initial levels were P.B.I. 5.2 gamma per cent, nitrogen excretion 6.4 mg./minute, sodium 112 μEq./minute, and potassium 48 μEq./minute. During the period of stress (lasting approximately an hour), increases up to 87 per cent (mean 50 per cent) in nitrogen excretion, up to 443 per cent (mean 109 per cent) in sodium excretion, and up to 114 per cent (mean 59 per cent) in potassium excretion were observed. Significant elevation of serum P.B.I. was observed in 7 instances. In 6 such studies on 5 hypothyroid subjects, mean initial levels were P.B.I. 4.6 gamma per cent, nitrogen excretion 4.5 mg./minute, sodium excretion 87 μEq./minute, and potassium 37 μEq./minute. During the period of stress increases up to 49 per cent (mean 21 per cent) in nitrogen excretion, up to 185 per cent (mean 69 per cent) in sodium excretion, and up to 45 per cent (mean 26 per cent) in potassium excretion were observed. Significant elevation of serum P.B.I. was not observed.

The differences in the increases in nitrogen and potassium excretion in the two groups were significant ($P < 0.05$) but the differences in the increases in water and sodium excretion were not ($P > 0.05$).

No differences from euthyroid subjects were observed in one adrenalectomized subject and one Addisonian subject maintained on cortisone and desoxycorticosterone acetate.

The findings suggest that the thyroid is exerting a "regulatory" role in the excretion of nitrogen and potassium during stressful life experiences in contrast to the "permissive" role of the adrenal cortex.

Studies of Pregnancy in Adrenal-Deficient Women. A. G. Hills, E. H. Venning, G. W. Webster and E. M. Richardson, Philadelphia, Pa. (Introduced by F. C. Dohan).

The occurrence of pregnancy in two women who because of severe hypertensive disease had been adrenalectomized (subtotally and totally respectively) permitted studies concerning the question of "adrenal-cortical like" activity in the adrenal-deficient pregnant woman. Urinary elimination of neutral 17-ketosteroids and of neutral reducing lipids (chemocorticoids) was markedly and progressively increased in both women from early in the second trimester until delivery. Biocorticoids (glycogenic corticoids) were excreted in the urine of both women during the latter half of pregnancy in quantities amounting to about four times the values observed during the non-pregnant periods.

The quantity of 17-ketosteroids and of biocorticoids excreted by these women in the latter half of pregnancy was in the general range of values observed at corresponding periods in normal pregnancy, and the biocorticoid excretion was of the order of magnitude encountered in Cushing's syndrome. In spite of these high values for urinary steroid excretion, both women were shown to be vulnerable to the development of acute adrenal insufficiency during the second and third trimesters of pregnancy; and in the one patient who required steroid therapy at all times, no decrease of hormone requirement accompanied pregnancy.

The conjunction of adrenal insufficiency with the very high values for the urinary steroids, especially the biocorticoids, indicates either 1) that pregnancy greatly increases hormone requirement or 2) that during pregnancy the urinary excretion of chemocorticoids, 17-ketosteroids, and biocorticoids fails to afford a roughly quantitative reflection of available (secreted plus exogenous) cortical hormone, owing to a presumptive alteration of the usual pathways of steroid metabolism resulting in augmentation of the proportion of secreted steroids and their metabolites eliminated in the urine. A definitive choice between these possible explanations is not warranted at present.

Some Aspects of Kidney Function in Hepatolenticular Degeneration (Wilson's Disease). Robert E. Hodges, Walter M. Kirkendall, Charles Schwartz and J. Burnette Wild, Iowa City, Ia. (Introduced by Horace M. Korns).

The cause of increased renal excretion of amino acids and copper in Wilson's disease is unknown. Uzman suggested that aminoaciduria causes increased copper excretion when oligopeptide compounds are in a chelation complex with copper. The aminoaciduria may result from competition between free amino acids and peptides for common tubular transport mechanisms.

We studied four patients with Wilson's disease and three normal persons with standard renal clearance methods using insulin and para-aminohippuric acid (PAH) in protein hydrolysate and determined renal clearances of copper and amino acid nitrogen. Renal extraction of PAH was measured in one patient with Wilson's disease.

Glomerular filtration was within normal limits in both groups of patients. Renal plasma flow in the Wilson's disease patients was approximately 2/3 of the normal. Renal extraction of PAH was 91 per cent in one patient with Wilson's disease. During protein hydrolysate infusions plasma levels of amino acid nitrogen rose and copper remained unchanged in both groups. Clearances of amino acid nitrogen rose equally in both groups. However, clearances of copper rose only in the Wilson's disease patients and averaged about three times pre-infusion rates.

These findings demonstrate a significant reduction in renal blood flow in this group of patients with Wilson's disease. With the normal glomerular filtration this suggests abnormal renal vascular resistance. The parallel rise in excretion of copper and amino acid nitrogen is in keeping with Uzman's hypothesis of competitive tubular reabsorption. The findings might also be explained by postulating a primary tubular defect.

The Role of Regeneration in the Morphogenesis of Experimental Fatty Cirrhosis. F. W. Hoffbauer,* Minneapolis, Minn.

The transformation of a fatty liver to cirrhosis has been studied in male rats (initial weight 100 grams) fed a

choline deficient diet. Five recognizable stages are: I, (10–15 days) centrolobular cells fatty. II, (15–30 days) all liver cells fatty, centrolobular fatty cyst disintegration underway. III, (30–60 days) periportal regeneration (normal hyperplasia). IV, (60–90 days) compression of centrolobular fatty cysts and formation of fibrous septa linking central veins. V, (90 days to death, 120–180 days) pathological regeneration—concentric nodular hyperplasia.

The manner of liver regeneration, initially of a normal order (stage III and IV) and later pathological in character (stage V), appears to determine the pattern of architectural alteration.

The situation in stage II is comparable to that of an animal subjected to partial hepatectomy, i.e. there is a marked deficiency in the number of *functioning* liver cells. The response is regeneration—normal hyperplasia. The multiplication of cells occurs about the small portal triads where the parenchyma is the least fatty. This periportal growth of new cells exerts pressure on the disintegrating fatty cysts (stage III) and, continuing, determines the location of septa composed of micromembranes (stage IV). The normal hyperplasia increases the size of the already large fatty liver. As long as it persists, the liver maintains a characteristic microscopic picture (fibrocirrhosis—normal spatial relationship, lobular pattern outlined by fibrous septa). The animal remains healthy.

There is a constant loss of functional tissue as newly formed cells become fatty. This provides a continuing intense stimulation for regeneration. Ultimately the orderly growth of new cells gives way to pathological regeneration (stage V). Individual concentric hyperplastic nodules appear and gradually increase in number. Normal regeneration apparently ceases and the growing nodules predominate. The microscopic picture (fibronodular cirrhosis) is one of disorganization; distortion and compression of the original liver tissue and especially the intrahepatic veins is apparent. During this stage the size of the liver usually decreases, the animal loses body weight and terminally exhibits effusions.

Effects of CO_2 Retention on Intracellular Acid-base Balance. DUNCAN A. HOLADAY, New York, N. Y. (Introduced by E. M. Papper).

Blood and thigh muscle samples were obtained from 15 anesthetized dogs before and after 90 minutes of normal respiration or induced hypoventilation with an oxygen enriched gas mixture. These methods of ventilation did not reduce arterial saturation to levels below the control in 14 experiments. pH, CO_2 content, oxygen content and capacity, hematocrit, chloride, sodium, potassium, and water content were obtained on whole blood and plasma; inorganic phosphate and lactic acid contents were obtained on plasma. Tissue chloride, sodium, potassium, water, and fat contents were determined. Tissue CO_2 content was analyzed by the method of Danielson and Hastings. Derived values included: venous blood, pCO_2, buffer base (Singer and Hastings nomogram), extracellular water and electrolyte contents, intracellular pH, water and electrolyte contents. Control animals exhibited insignificant changes in the above values. Increases in pCO_2 betwen 74 and 138 mm. Hg maintained for 90 minutes resulted in an average reduction in plasma pH of 0.55, and a reduction in buffer base of 15.6 meq./L. of blood. The mean increase of blood bicarbonate was only 2.8 mM./L. Intracellular pH was reduced to the same extent as the plasma pH. The bicarbonate and sodium and potassium contents of cells remained essentially unchanged. If a buffer capacity similar to that of blood can be ascribed to cellular proteins, then decrease in pH was probably accompanied by the appearance of significant amounts of intracellular acids, as yet unidentified. These changes are also accompanied by a decrease of salt and water in the blood and an increase of salt and water in cells. Intermediate pCO_2 elevations (+30 to +57 mm. Hg) result in similar alterations of a proportionally smaller magnitude.

Biochemical Determination of Blood and Urine Catechol Amines as a Measure of Sympathoadrenal Activity in Hypertension. S. W. HOOBLER,* A. AGREST and R. J. WARZYNSKI, Ann Arbor, Mich.

By the method of Weil-Malherbe (Bioch. J. **51**: 311, 1952), it was possible to detect increases in the level of blood catechol amines under conditions of environmental stress in some hypertensive patients and a regular decline in these compounds when sympathoadrenal activity was suppressed by ganglionic blocking drugs. In two patients, stressful interviews raised the level of blood catechol amines from 1.6 to 3.1 and from 4.1 to 5.4 mcg./liter respectively.

In all seven patients treated with ganglionic blocking agents, a decline in blood level was noted. The mean value before treatment was 2.7 mcg./liter and after treatment was 0.47 mcg.

The decline in blood catechols was not proportional to the degree of reduction of blood pressure, and did not regularly occur after supradiaphragmatic sympathectomy ($D_8 - D_{12}$). Twelve normal patients, 2 hyperthyroid, 17 "stable" and 18 labile hypertensive individuals showed no significant difference in resting blood catechol levels.

The method of Weil-Malherbe was modified for urine analyses by removing the catechols on an alumina column at pH 8.4, eluting with 0.2N acetic acid, concentrating to dryness and separating from each other by paper chromatography. In 6 normals, the average 24-hr. secretion of noradrenaline was 104.7 mcg./24 hrs., adrenaline was 33.2 mcg. and hydroxytyramine 87.6 mcg. Fourteen hypertensive subjects showed no significant differences.

In one case of pheochromocytoma, the urinary catechols were noradrenaline 2,534, adrenaline 160 and hydroxytyramine 1462 mcg. per 24 hrs.

In conclusion there appears to be an approximate correlation between the blood levels of catechol amines and sympathoadrenal activity. On the basis of this measurement, no increase can be noted in resting hypertensive subjects. Their response to stress remains to be studied.

24-hour urine excretion of the individual catechol amines can now be measured with fair accuracy, and does not appear altered in essential hypertension.

Blood (Red Cell) Volume Measured by Carbon Monoxide and Radiochromium Methods. JAMES HOPPER, JR., NORMAN NOMOF, ELLEN BROWN, REIDAR WENNESLAND and KENNETH G. SCOTT, San Francisco, Calif. (Introduced by T. L. Althausen).

CO and Cr^{51}-available spaces were determined simultaneously under resting conditions in 6 healthy subjects and 29 patients with various diseases. In all but 2 of the 35 subjects, the CO space was greater than Cr^{51} space, and the average discrepancy was 16%. The difference in spaces was of the same magnitude in disease states as in health. Distribution of both CO and Cr^{51}-tagged cells was complete within 20 minutes, and Cr^{51}-space remained nearly constant for 24 hours. It is concluded that (a) the entire contents of the vascular compartment are reached by these indicators, and (b) CO is distributed to a space which is about 16% larger than intravascular red cell volume. Since the CO reaches its maximum volume of distribution during the first 20 minutes of re-breathing, it is assumed that it becomes bound by substances having affinity for CO similar to that of the hemoglobin in circulating cells. Experiments are in progress to determine the areas of the body where such combination occurs. Relative concentration of CO and Cr^{51} is determined in various tissues of the rabbit, including bone marrow. Differential measurements of CO and Cr^{51} spaces may prove to be a means of estimating stores of non-circulating hemoglobin-like substances.

Clinical and Sub-Clinical Responses to Poliomyelitis Infection in Families. D. M. HORSTMANN* and R. W. MCCOLLUM, New Haven, Conn.

The present study is concerned with factors governing the incidence of infection among familial and other close contacts of poliomyelitis cases. Attention was focussed on the correlation of infection (or its absence) with immune status, and on the relation of age to clinical manifestations of the disease. Such a study is now feasible because of the introduction of tissue culture techniques into poliomyelitis research.

The group studied consisted of 32 index cases and their 91 household and daily contacts. Twenty-eight of the contacts were found to be infected with Type 1 poliomyelitis virus; 13 of these had symptoms compatible with the "minor illness," and 15 were asymptomatic. When the antibody status of these who failed to become infected was determined, it was found that a number already possessed Type 1 antibodies, and could therefore be considered immune. When only susceptibles were considered, the infection rates were very high indeed: of 73 household and daily contacts under 15 years of age, 29 were susceptible; of these, 26 (90%) were infected. In contrast, of the 18 in the age group 15 years old and over, 10 were susceptible, and only 2 of these (20%) were excreting virus.

Although infection rates were uniformly high through 0–14 years, the clinical response in different age groups varied. A trend toward milder infections and less paralysis was noted in infants and young children when clinical manifestations of the 60 infected individuals (32 cases and 28 contacts) were analyzed. The ratio of inapparent to apparent infections was about 1:1, in contrast to the estimated figures of 100:1 for the general population.

Cardiac Output by Body Surface Counting of I^{131} Human Serum Albumin. REX L. HUFF,* D. D. FELLER and GEORGE BOGARDUS, Seattle, Wash.

A new and simple technique for measuring cardiac output has been devised employing body surface counting following a single intravenous injection of I^{131} Human Serum Albumin (HSA). The simplicity, reliability, and rapidity of the method give great advantages over other methods. It can be carried out in a physician's office and can be used to measure the cardiac output of exercising individuals.

The injection of I^{131} HSA is made into the antecubital vein of man or the femoral vein of dogs and the counting rate recorded from a narrow-angle scintillation counter placed on the skin between the first and second ribs at the left parasternal line. Count rates as a function of time are obtained which are similar to those obtained by direct arterial blood sampling. The area under the extrapolated curve, measured as in the direct eye or radioisotope procedure, is divided into the equilibrium counting rate. The dimension of the cardiac output thus obtained is in terms of a multiple or fraction of the subject's blood volume per unit time. Conversion to liters/min. is made by taking several venous blood samples for *blood volume* determination.

Validity and reliability of the technique are affirmed by the fact that measured cardiac indices of hospitalized patients agree with those expected from the history and physical findings of the patient. Normal individuals have normal values. Furthermore, simultaneous cardiac indices measured by the Direct Fick Method agree favorably with this *in vivo* isotope method.

Up to three separate determinations may be performed at one visit within a half hour period. In some cases, further tests are limited by the radiation tolerance doses for I^{131}; however, repetition is permissible at a later date.

A Study of the Physiologic Closure of the Ductus Arteriosus in Newborn Infants. HERBERT N. HULTGREN, FREDERIC L. ELDRIDGE and MARY E. WIGMORE, San Francisco, Calif. (Introduced by Lowell A. Rantz).

The exact time of functional closure of the ductus arteriosus in the normal human infant has never been determined, although animal experiments have suggested closure immediately after birth.

To determine this, arterialized capillary blood samples were obtained from the right hand and foot of 33 normal

newborn infants and analyzed for oxygen content and capacity.

31 of the 33 infants showed right hand oxygen saturations of 90% or more, indicating that true arterial blood was obtained. Up to 3 hours after birth, the oxygen saturation of blood from the foot was significantly lower than that from the hand in 9 of 12 cases studied, indicating the presence of a veno-arterial shunt through the ductus arteriosus. Calculations in these cases indicate the ductus was contributing from ¼ to ½ of the total blood flow in the descending aorta. In infants 3–72 hours after birth, 7 of 15 cases showed evidence of a veno-arterial shunt through the ductus. None of the 6 infants over 3 days of age showed any significant difference in saturation between right hand and foot.

These findings indicate that the ductus arteriosus is patent, that a veno-arterial shunt occurs in most infants under the age of 3 hours, and that this may persist in a significant number of infants up to the age of 3 days. In those infants showing no difference in hand-foot saturations and in infants over 3 days of age, either the ductus had closed or the shunt had become arterio-venous.

Low oxygen mixtures (12%) were administered to 4 infants showing no evidence of veno-arterial shunt. Significant differences in hand-foot saturations were produced indicating either re-opening of a closed ductus or reversal of flow through a patent ductus due to increased pulmonary vascular resistance.

The Effect of Cortisone and Hydrocortisone on the Peripheral Utilization of Thyroid Hormone. SIDNEY H. INGBAR and NORBERT FREINKEL, Boston, Mass. (Introduced by Maxwell Finland).

Changes in iodine metabolism have been described during induced hyperadrenocorticism or stressing environmental manipulations. Studies were designed to investigate the mechanisms whereby these changes are induced.

By a method designed to minimize errors resulting from mixing phenomena, thyroxine utilization rates were determined in 9 patients on constant diets before and during the administration of cortisone or hydrocortisone. Thyroxine space was calculated daily for 8–16 days as the quotient of the residual radiothyroxine (administered dose minus fecal, urinary, and thyroidal collection of radioiodine) and the plasma radiothyroxine concentration. After distribution equilibrium had been achieved, as evidenced by constancy of the calculated space, the fractional daily turnover was determined from the exponential slope of the plasma concentration. Utilization rate was calculated as the product of the plasma PBI and the daily volume turnover (fractional turnover × space).

In 4 patients with primary myxedema and in 3 in whom thyroid function had been abolished by exogenous hormone, alterations in pituitary and thyroidal function were eliminated as experimental variables. Daily doses of 75–200 mg. of steroid in these patients and of 25 mg. in 2 patients with panhypopituitarism failed to change utilization rates or PBI's significantly. In the latter patients cortisone did not directly suppress thyroidal function, since calculated values of the daily hormone production were also unchanged.

These data suggest that during periods of dietary control, rates of thyroxine utilization are not conditioned by the availability of 11-17-oxygenated steroids. Hence other mechanisms must be invoked to explain changes in iodine economy which follow certain noxious stimuli.

Bacteriophage Typing of Staphylococci in Clinical Epidemiology with Observations on Antibiotic Resistance. GEORGE GEE JACKSON and MARK H. LEPPER, Chicago, Ill. (Introduced by Harry F. Dowling).

Clinical investigation of infections caused by staphylococci has been impeded by the lack of a suitable means of classifying strains within the species except by broad biologic characteristics. Using 20 staphylococcic bacteriophages, 2085 strains of coagulase positive and coagulase negative staphylococci isolated from 795 persons have been tested for patterns of lysis. One-hundred-twenty-nine patterns of strong lysis were observed among 541 coagulase positive strains, and these enabled the identification of specific strains with a high probability of validity.

The bacteriophage reactions permitted classification of typeable strains of staphylococci into 4 broad groups according to the grouping of phages suggested by Williams on the basis of the association of lysogenic reactions, and 4 additional intermediate groups. Most of the broad phage groups were represented among cultures taken from different anatomical sites but particular groups showed a statistically significant increase or decrease in the frequency of occurrence in the respiratory tract. Ninety per cent of the strains isolated from patients with staphylococcic enteritis in 3 hospitals could be assigned to 2 phage groups. The distribution of the phage groups among patients, their contacts, and hospital personnel was not in the same proportion as their overall representation. Observations were made on the exchange of strains in persons within each of these population categories.

The development of resistance to the action of penicillin, chlortetracycline and erythromycin does not alter the phage reaction of the strain and statistically significant differences in the number of sensitive and resistant strains were observed among the different phage groups for each of the antibiotics tested.

The Effect of 17 α-Hydroxyprogesterone upon the Urinary Excretion of 17-Ketosteroids and "Pregnanediol Complex" and its Possible Role in Congenital Adrenal Virilism. JOSEPH W. JAILER,* JAY J. GOLD, SEYMOUR LIEBERMAN and RAYMOND VAN DE WIELE, New York, N. Y.

Previous investigations have shown that the administration of ACTH to patients with congenital adrenal virilism results in no increase in the excretion of formaldehydogenic steroids; no retention of sodium; and no fall in eosinophils (Wilkins et al.; Bartter and Albright; Jailer et al.), but does result in a marked rise in the 17-ketosteroid and "pregnanediol complex" excretion. Con-

sequently, one can postulate that the adrenals of these patients have difficulty in synthesizing hydrocortisone, and elaborate instead steroids which are precursors of the 17-ketosteroids and the "pregnanediol complex." Hechter, Pincus, and others have demonstrated that progesterone can be hydroxylated by surviving adrenal tissue at the C-11, 17, 21 positions to form hydrocortisone. One of the intermediary steroids formed in this biosynthesis is 17 α-hydroxyprogesterone. This steroid had been previously isolated from adrenal tissue by North and Pfiffner, and has been shown to be androgenic when administered orally to chicks. 17α-hydroxyprogesterone has been administered orally to 8 patients; 2 patients with Addison's disease, 2 patients with hypopituitarism, and 4 patients with the adrenogenital syndrome whose 17-ketosteroid excretion was being suppressed with cortisone. In all cases a rise in the 17-ketosteroid and "pregnanediol complex" excretions occurred. Chromatographic separation and infra-red identification of the 17-ketosteroid complex revealed the presence of androsterone, etiocholanolone, and 17-hydroxypregnanolone which are urinary metabolites usually found in the adrenogenital syndrome. One can speculate that in adrenal virilism an enzymatic block exists which prevents the complete hydroxylation (probably at C-11 and C-21) of steroidal precursors of hydrocortisone. This may result in the secretion of excessive amounts of 17α-hydroxyprogesterone. Since 11-oxygenated 17-ketosteroids are found in high concentrations in the urine of some patients with virilism, it is possible that the block in hydroxylation in these patients occurs only at the C-21 position, and this may lead to the excessive secretion by the adrenal of "11β, 17α-dihydroxyprogesterone" or 21-desoxyhydrocortisone which then is metabolized to 11-oxygenated ketosteroids.

Studies on the Mechanism of the Anemia of Liver Disease. James H. Jandl, Boston, Mass. (Introduced by W. B. Castle).

Red cell survival studies on 20 patients with chronic alcoholism, hepatic cirrhosis, and macrocytic anemia revealed a hemolytic process proportional in severity to the degree of anemia. The calculated daily hemoglobin production and the total urobilinogen excretion of these patients both averaged 1.5 times normal, and ranged up to 3 times normal. An increase of hemoglobin values, associated with persistently elevated reticulocyte levels and increased numbers of nucleated red cells in the marrow, occurred spontaneously in most patients despite little or no improvement in red cell survival. The rate of hemolysis was not influenced by cortisone. Except for initial large red cell volumes and except for loss of osmometric function together with increase in mechanical fragility, developing after 24 hours of sterile incubation, the patients' red cells were not measurably abnormal. Red cell "flattening" as seen in acute hepatitis and obstructive jaundice was not generally present. Serum iron and serum iron-binding capacity levels were both significantly reduced in the presence of normal or excessive deposits of hemosiderin in the liver and bone marrow. Abstinence from alcohol was consistently followed by a rise in reticulocyte levels, occurring independently of diet and bed rest, suggesting that ingested alcohol suppresses the marrow response to the anemic state.

Susceptibility of Cells to Viral Infection. Karl M. Johnson and Herbert R. Morgan,* Rochester, N. Y.

Viruses exhibit tissue tropisms and vary widely in their ability to grow in different tissues *in vitro* as well as *in vivo*, but factors responsible for this specificity remain largely undefined.

A model system, using psittacosis virus and chick embryo tissue, was devised to study this problem *in vitro*. Cultures of tissue lose their capacity to support virus multiplication when maintained in a nutritionally deficient culture medium of glucose and mineral salts. Virus production can be initiated subsequently in these cells by the addition of a chemically defined culture medium (No. 199 of Parker) containing numerous amino acids, vitamins, and accessory growth substances.

Furthermore, it has been shown that virus can enter these cells made deficient prior to its introduction in spite of the fact that multiplication fails to occur subsequently. Stimulation of virus growth occurs in the absence of cellular proliferation which can be prevented by the addition of colchicine.

Studies using various components of the chemically defined medium indicate that (1) fat-soluble vitamins, (2) certain purines and pyrimidines, and (3) ATP are *not* essential for reversal of the deficient state of the cells while (1) amino acids, (2) B vitamins, and (3) biological reducing agents are required for initiation of viral multiplication.

These studies of the nutritional requirements of psittacosis virus in deficient cells provide information concerning the factors which determine whether a tissue will support growth of a given virus, i.e., tissue tropism.

Studies on the Pulmonary Circulation: Effects of Anoxia, Exercise, and Hexamethonium in Patients with and without Thoracic Sympathetic Denervation. Walter E. Judson, William Hollander and Julia G. Arrowwood, Boston, Mass. (Introduced by Robert W. Wilkins).

Of six patients who had had a transthoracic sympathectomy (D-1 to at least D-5) for arterial hypertension and tachycardia or for Raynaud's Disease, only three were effectively denervated as indicated by the presence of Horner's syndrome, by the absence of the peripheral arterial blood pressure "overshoot" to the Valsalva maneuver, and by a relatively fixed pulse rate during exercise. These three patients during the inhalation of 10 per cent oxygen for at least 15 minutes had no change in pulmonary arterial pressures, in cardiac output (Fick), or in pulmonary "capillary" pressure whereas the three other patients with incomplete denervations had rises in pulmonary arterial pressure without changes in cardiac output. In all cases the cardiovascular responses to exercise were normal. After intravenous hexamethonium the

pulmonary arterial pressures usually fell without consistent changes in the calculated pulmonary arteriolar resistances.

A thoracic epidural or "differential" spinal block (procaine) was performed on six patients, two of whom were in congestive heart failure. On the basis of Horner's syndrome, pulse rate response to exercise, blood pressure response to the Valsalva maneuver, and sensory changes, only three patients had effective sympathetic denervation from at least D-1 to D-5. In these three patients, prior to the spinal block procedure there was a rise in pulmonary arterial pressure during the inhalation of 10 per cent oxygen (primarily as a result of an increase in the calculated pulmonary arteriolar resistance), whereas following the block there was no change in pulmonary arterial pressure, in cardiac output, or in pulmonary "capillary" pressure during anoxia.

Ten patients with compensated arterial hypertension, eight with mitral stenosis, and four with cor pulmonale were exercised and given inhalations of 10 per cent oxygen both before and after intravenous hexamethonium. After hexamethonium there was a fall in systemic arterial pressure, usually associated with a fall in pulmonary arterial pressure and pulmonary arteriolar resistance (without a change in cardiac output). However, when the cardiac output decreased after hexamethonium, the pulmonary arteriolar resistance increased, and it also increased when the lowered peripheral arterial pressure was restored to normal by intravenous norepinephrine. After hexamethonium an increase in pulmonary arterial pressure and arteriolar resistance still occurred during anoxia. Following hexamethonium patients developed less rise in pulmonary arterial pressure during exercise than they had before hexamethonium. Likewise they had a fall in calculated pulmonary arteriolar resistance during exercise instead of a rise as before hexamethonium.

It is concluded:

(1) Anoxia induces a rise in pulmonary arterial pressure through the activity of the thoracic sympathetic nervous system.

(2) Hexamethonium in the dosage used caused changes in pulmonary circulation apparently secondarily to the changes it produced in the systemic circulation and not through direct thoracic sympathetic paralysis.

Alterations in the Nature of the Adrenal Secretion Induced by the Prolonged Administration of ACTH to Rabbits. EDWARD H. KASS,* OSCAR HECHTER, I. ALDEN MACCHI and THOMAS W. MOU, Boston, Mass.

Differences between the effects of adrenocorticotropin and individual steroids of adrenal origin are becoming increasingly apparent. The adrenal glands of rabbits were reported to secrete predominantly corticosterone, yet this steroid did not significantly affect the structure and ribonucleic acid concentrations of rabbit lymph nodes, and depressed antibody production but slightly. ACTH, as well as hydrocortisone and cortisone, exerted marked effects on the lymph nodes and on antibody production. The paradoxical response to ACTH led to investigation of its effect on the adrenal steroid content of adrenal vein blood.

Corticosterone was found to be virtually the sole steroid component of adrenal vein blood of untreated control rabbits. There was no detectable hydrocortisone or cortisone. A single dose of ACTH did not significantly change the findings. However, when ACTH was given for one week, hydrocortisone was detected in a ratio to corticosterone of 1:2. When ACTH was given for 21–28 days, hydrocortisone became the predominant steroid, in a 4:1 ratio over corticosterone, and several unidentified trace components, not previously present, were demonstrable. The effects of chronic trauma are under investigation.

Thus, the qualitative nature of the adrenal secretion is not necessarily fixed genetically. There are probably two different actions of ACTH; the first is rapid and direct, and increases the steroid output of the adrenal gland without altering the nature of the secretion. The second effect is delayed, and may involve changes in the chemical nature of the secretory product, with different physiological and pathological effects.

Cardiac Output and Central Volume in Heart Disease as Determined by the Dye Dilution Technique. ALBERT KATTUS, ARTHUR RIVEN, AARON COHEN and GILBERT SOFIO, Los Angeles, Calif. (Introduced by Samuel H. Basset).

The Hamilton dye dilution technique for the determination of cardiac output has been used to study the circulation in a variety of clinical states and in a group of normal subjects. From the slope of the down-stroke of the semi-logarithmically plotted concentration curves the central volume was calculated by the method of Newman. This central volume is believed to be closely related to the pulmonary blood volume.

Subjects were studied at rest in the post absorptive state. Cardiac output is expressed as the Cardiac Index (liters per square meter of body surface area) and central volume as Central Volume Index (cubic centimeter per square meter of body surface area). Average values for Cardiac Index (CI) and Central Volume Index (CVI) were determined in various categories.

In 7 normal subjects average CI was 3.5 liters per minute and CVI was 659 cc. In 23 pre-operative mitral stenosis patients CI was 2.4 and CVI was 481 cc. After commissurotomy, CI was 2.5 but CVI had increased to 543 cc. In 10 pre-operative aortic stenosis patients CI was 2.8 and CVI was 557. After valvulotomy CI increased to 3.5 but CVI did not change.

Patients with right ventricular failure (elevated venous pressure and edema) due to coronary arteriosclerosis and high output cor pulmonale had large CVI (723 to 1310 cc.). Those due to hypertension, aortic insufficiency, thyrotoxicosis and low output cor pulmonale had CVI less than the normal (392–582 cc.).

The studies indicate that congestive heart failure may be associated with a pulmonary blood volume less than normal.

The Correlation of Human Gastric Potentials with Gastric Secretion as Measured by the Electrogastrogram. IRWIN KATZKA, FREDERICK JACKSON, JR. and HENRY M. LEMON, Boston, Mass. (Introduced by Franz J. Ingelfinger).

The electrogastrogram has been studied by others to diagnose gastric disease but the physiologic significance of the intragastric potential so measured is not established. Investigations restricted to experimental animals have suggested that these potentials are produced by (1) the degree of acidity of gastric juice and (2) the electrical energy involved in the production of hydrochloric acid. We have carried out studies in man and have shown that these potentials are related to the process of secretion rather than change in concentration or acidity of gastric juice.

Using intragastric saline electrodes, calomel half cells and a new commercially available potentiometer, we recorded the potentials in the fasting stomach and following the administration of histamine or insulin in 45 normal subjects. With histamine, the potential increased rapidly from the fasting level and reached its peak before the maximum increase in gastric acidity occurred. With insulin, the rise in potential began 25–30 minutes later, correlating with hypoglycemia and again preceded maximum acidity. Gastric potentials were not increased by the introduction of acids, alkalies or gastric juice into the stomach. Furthermore, histamine produced no potential change from the stomachs of patients with pernicious anemia or from the esophagus or duodenum or skin of normals. In 3 out of 5 cases, intravenous Banthine decreased the potential independent of the changes in gastric pH.

The findings to date suggest that electrical potential is related to gastric function but it is independent of the character of gastric juice. The changes in electrical activity induced by the aforementioned drugs may be related to changes in the physicochemical activity of the gastric mucosa in the process of secretion and/or changes in the vascularity of the organ.

The Excretion of Acid Mucopolysaccharides in Normal Human Urine. GRACE P. KERBY,* Durham, N. C.

The amount of glucuronic acid excreted in the form of acid mucopolysaccharides in the urine of normal humans has been investigated as an approach to studying the metabolism of these substances. The fraction has been obtained from urine by the method described by Astrup.

The mean 24-hour excretion of acid mucopolysaccharides was 3.0 mg ± 0.6 (S.D.) and 4.9 mg ± 1.3 (S.D.) in white females and males, respectively. The difference between the two values was significant ($p = < 0.01$). Prostatic massage in one patient did not increase the amount of the material in urine. The material was present in full amount in urine obtained from the renal pelvis of one patient and thus was not derived to significant degree in this patient from structures lower in the urinary tract.

The urinary excretion of acid mucopolysaccharides by humans was markedly increased by the ingestion of glucurolactone (5 gm daily for several days).

Chromatographic studies of a further-purified fraction of acid mucopolysaccharides from normal human urine were done by a method developed during the present investigation. The major component of the fraction had the same R_f value as chondroitin sulfate and produced a single spot when mixed with chondroitin sulfate, using a single solvent system.

The present information was sought to provide a background for the further study of certain metabolic disorders and hypersensitive states. Such studies are now in progress.

Alcohol, an Effective Inhibitor of the Release of Antidiuretic Hormone (ADH). CHARLES R. KLEEMAN, MILTON E. RUBINI and EZRA LAMDIN, New Haven, Conn. (Introduced by John P. Peters).

To evaluate the potential therapeutic effect of alcohol in conditions characterized by water retention, its action on the release of ADH in response to changes in volume, effective osmolarity and other variables has been studied. In contrast to numerous factors that enhance the activity of ADH, only alcohol and water are known to inhibit it.

Semirecumbent normal men, under various conditions imbibed 120 cc. of bourbon whiskey. Electrolytes and freezing point depressions of serum and urine were measured. Urine flow (cc./min.) was divided into two fractions: Osmolar clearance $= \frac{\text{Urine osmolarity}}{\text{Plasma osmolarity}} \times$ Urine flow; and Free water clearance = Urine flow − Osmolar clearance. Controls without alcohol were made in each series.

After alcohol free water clearance and urine flow rose, while excretion of sodium, potassium and chloride fell and a mild metabolic acidosis developed.

When a maximum urine flow was produced by sustained loads of water, alcohol had no effect on urine flow or free water clearance. It had a similar negative effect on a patient with diabetes insipidus.

The antidiuretic effect of hypertonic salt solution during maximal water diuresis was prevented by alcohol, which instead caused urine flow and free water clearance to rise further. Solute without water was, therefore, reabsorbed in a region of the tubules in which ADH presumably acts.

Prior alcohol imbibition minimized the antidiuretic effect of venous congestion of the lower extremities although excretion of solutes decreased.

The effect of alcohol was modified if it was given *subsequent* to the stimulation of ADH by hypertonic salt or venous congestion.

Alcohol did not improve the impaired excretion of water in Addison's disease.

Conclusions: Alcohol acts upon the supraoptico-hypophyseal system to block *all* stimuli provoking the release of ADH. This inhibitory action is being investigated in

clinical states in which excessive activity of ADH has been hypothesized.

Influence of Antibiotic Treatment on the Rate of Acquisition by Patients of Multiple Drug-Resistant Phage Group III Staphylococci Indigenous to a Hospital. VERNON KNIGHT, New York, N. Y. (Introduced by Walsh McDermott).

At Bellevue Hospital, it was possible to determine the relative rates of acquisition of "hospital strains" of staphylococci by newly hospitalized patients receiving various therapeutic regimens. The determination was possible because a large majority of "hospital staphylococci" were of group III phage patterns resistant to penicillin, the tetracyclines, and streptomycin, in contrast to a negligible percentage of such strains in admission cultures. In the study, acquisition rates were estimated for 65 patient carriers treated with penicillin, the tetracyclines, or no antibiotics.

Practically all tetracycline resistant strains were "hospital staphylococci," and they constituted about *one-tenth* of strains obtained at hospital admission. *One-half* of admission strains were penicillin resistant, the excess over one-tenth being strains not classifiable as "hospital staphylococci."

The rate of acquisition of "hospital staphylococci" was greatest among patients treated with tetracyclines, rising to 86 per cent in the 4–5-day period. It was second greatest among strains exposed to penicillin, not rising appreciably from admission values until the second week. The linkage between tetracycline and penicillin resistance among "hospital staphylococci" led to the paradoxical phenomenon that the rate of increase in *penicillin* resistance was greatest among strains from patients treated with tetracyclines. The rate of increase of "hospital staphylococci" was least among untreated patients.

Thus, there was demonstrated a close correspondence between the rate of acquisition of drug resistant "hospital strains" by staphylococcal carriers, and the degree of susceptibility of the strains they carried on admission to the agents used in treatment. It is postulated that the extent of elimination of susceptible strains by treatment determines the degree to which replacement with "hospital strains" may occur, and this hypothesis is supported by the observation that there is a low rate of acquisition of such strains by patients who are untreated.

Studies on Antibodies Eluted from the Erythrocytes in Autoimmune Hemolytic Anemia. ZACHARIAS D. KOMNINOS and MUZAFFER AKSOY, Boston, Mass. (Introduced by William Dameshek).

Antibodies eluted from the red cells in autoimmune hemolytic anemia were studied with respect to their thermal range of activity, ability to produce a specific type of Coombs' serum and reactivity with isolated fractions of red cell stroma. Twenty-nine eluates were studied from 11 patients with "idiopathic" and 4 with "symptomatic" hemolytic anemia. Dissociation of antibody from the red cells was achieved by exposure to low pH, high salt concentration or high temperature.

Results: The titer of the eluates, even when cold hemagglutinins were present in the serum, was distinctly lower at 3° than at 37°. Agglutination was also weaker in the cold and took longer to develop. Absorption experiments indicated that erythrocytes could remove more antibody at 37° than at 3°. By injection of eluates into rabbits, Coombs' sera were produced ranging in titer from 1/4000 to 1/10,000. Coombs' sera of approximately the same titer were also obtained by injection of coated red cells from patients with autoimmune hemolytic anemia. These various sera were non-specific as they could agglutinate erythrocytes sensitized with iso(Rh)-antibodies equally well. Normal human red cell stroma was extracted with $NaHCO_3$ (fraction I) and with acetone (fraction II). Only fraction I could neutralize the antibody in all eluates tested. Boiling of this fraction destroyed its antibody neutralizing ability.

Conclusions: The antibody attached to the erythrocytes in autoimmune hemolytic anemia was of the warm variety in all cases tested. Coombs' sera obtained by injection of eluates or coated red cells into rabbits were not specific for auto-antibodies. The red cell antigen with which the eluted antibodies reacted was extractable with $NaHCO_3$ and heat labile, indicating its probable protein nature. The eluted antibodies of autoimmune hemolytic anemia have many of the characteristics of "immune" antibodies.

Influence of Certain Diseases on the Absorption of Vitamin B_{12} from the Gastrointestinal Tract. JULIUS R. KREVANS, C. LOCKARD CONLEY* and MARIE SACHS, Baltimore, Md.

Vitamin B_{12} labelled with Co^{60} was administered orally to normal individuals and patients with various diseases. Stools collected for 6 days were dried, pulverized, and the gamma radiation measured under the same conditions used for the measurement of the radioactivity of the B_{12} administered.

In 16 studies on normal individuals administration of 0.1–0.5 micrograms of labelled B_{12} was followed by recovery of 10–47 per cent of the radioactivity in the stool. Within this range there was no relationship between the amount of B_{12} administered and the per cent recovered. With a dose of 1.0 microgram as much as 60 per cent of the radioactivity was recovered, and with 5.5 micrograms, more than 70 per cent.

In 23 studies on patients with diseases unrelated to pernicious anemia, administration of 0.1–0.5 micrograms of labelled B_{12} was followed by recovery of 10–45 per cent. This group included individuals with achlorhydria, liver disease, and anemia unrelated to B_{12} deficiency. One 21 year old male with hepatitis and normal gastric acidity in two studies excreted 67 and 68 per cent.

Eight patients with pernicious anemia excreted more than 60 per cent of the administered radioactivity of an administered dose of 0.5 micrograms. One patient who appeared to have typical pernicious anemia excreted less

than 35 per cent in two tests. Further study by the technique of Schilling indicated apparently normal B_{12} absorption in this patient.

One of 2 patients with total gastrectomy and 2 of 3 with sprue were found to have impaired absorption.

One patient with megaloblastic anemia, sub-acute combined degeneration, normal gastric acidity, and multiple diverticula of the jejunum demonstrated markedly impaired absorption which became normal during the administration of aureomycin. Terramycin failed to produce normal absorption in a patient with pernicious anemia.

The Metabolism of Intravenously Infused C^{14} Labelled Cholesterol in Man. GEORGE S. KURLAND, JUDITH L. LUCAS and A. STONE FREEDBERG,* Boston, Mass.

Preliminary observations of the metabolism of C^{14} labelled cholesterol following intravenous infusion have been made in 3 euthyroid patients with angina pectoris. C^{14} cholesterol, labelled in the 4 position in Ring A, was dissolved in saline with Tween 20. Aliquots of plasma, red blood cells and stools were analyzed at frequent intervals for 13 to 110 days. The specific activity (S.A.) of cholesterol in various tissues was determined in one patient who expired 15 days after the infusion.

Following a period of rapid decrease in radioactivity, the disappearance of labelled plasma cholesterol was not a simple exponential function but consisted of multiple components. The S. A. of RBC cholesterol rose rapidly and reached equilibrium with plasma free cholesterol 7–14 hours after infusion. The S. A. of the plasma total cholesterol, reflecting the radioactivity of the plasma cholesterol esters, reached equilibrium with plasma free cholesterol 4–7 days after infusion. In the period immediately thereafter, the T½ of the RBC cholesterol, plasma free cholesterol, and total plasma cholesterol was 10.8 to 13 days. Subsequent disappearance rates were progressively slower; in one patient, for example, the S. A. of free cholesterol on day 110 was 84% that on the 80th day after infusion. A "readily exchangeable cholesterol pool," determined by isotope dilution on day 7 and corrected for stool excretion, was 26 to 55 grams in the 3 patients. Daily fecal C^{14} radioactivity excretion was approximately 3% of the administered C^{14} during the first 9 days after infusion; thereafter, a marked decrease in fecal radioactivity occurred. Fecal radioactivity was found in the sterol, fatty acid, and bile acid fractions.

At post mortem examination, in Case 2, the S. A. of liver cholesterol was the same as that of the plasma. Progressively lower specific activities were found in muscle, skin, fat, and atherosclerotic vessel, respectively.

The data are consistent with the hypothesis that the labelled carbon of the cholesterol molecule has been reutilized in the formation of new cholesterol.

The Diurnal Variation in Adrenocortical Secretion. JOHN C. LAIDLAW, DALTON JENKINS, WILLIAM J. REDDY and THEODOR JAKOBSON, Boston, Mass. (Introduced by George W. Thorn).

The diurnal variation in urinary 17-hydroxycorticoid excretion has been studied on one or more days in 75 normal subjects. The pattern is almost uniformly characterized by a higher level of excretion between 7 a.m. and 7 p.m. than during the overnight period. The fall in 17-hydroxycorticoid excretion in the early evening does not usually persist until the following morning but is frequently succeeded by a rise in urinary levels around 3 a.m. Further confirmation of the predominance of the daytime excretion of 17-hydroxycorticoids was obtained from daily studies carried out over a 30-day period in three normal subjects; on only four occasions did the overnight excretion of 17-hydroxycorticoids exceed that of the day.

That the diurnal variation in 17-hydroxycorticoid excretion reflects predominantly an alteration in adrenal cortical secretion is suggested by experiments conducted on patients with Addison's disease given a maintenance dose of hydrocortisone (1–2 mg. per hour) by continuous intravenous infusion over a 24-hour period. In these patients, there was no predominance of day time excretion over that observed during the night.

Investigations of the mechanisms responsible for the diurnal variation in adrenal cortical secretion have indicated that the characteristic cycle persists despite enforced inactivity in bed, a reversal of the normal working schedule (night workers), a 24-hour period of starvation, a 24-hour period in which feedings were given every four hours and a reversal of the normal daytime predominance in urine output. The administration of ACTH during the night, however, leads to a reversal of the diurnal cycle. Further studies are being carried out in infants to determine the age at which the characteristic rhythm of adrenal cortical secretion becomes manifest.

These studies suggest that there is a basic diurnal regulation of adrenal cortical secretion, which is relatively resistant to changes in extrarenal environment.

The Metabolic Effects of Progesterone in Man. RICHARD L. LANDAU,* DELBERT M. BERGENSTAL, KATHLEEN LUGIBIHL and MARY E. KASCHT, Chicago, Ill.

It has been conclusively demonstrated that despite its restricted anabolic influence on the uterus and breast, progesterone, in approximately physiological amounts, promotes a general catabolic process. In three women and a man with apparently normal adrenal function 50 mg. per day of progesterone (intramuscular) induced a daily nitrogen loss which averaged 1–2 gm. and persisted as long as the hormone was given (maximum 30 days). Uric acid excretion rose slightly, but no other indicators suggested an adrenal corticoid influence. Free urinary alpha amino acids and 17-keto-steroids were not increased, and circulating eosinophils rose. A moderate elevation in urinary sodium and a slight chloruresis were observed during the first four to six days of progesterone administration, and both elements were retained during the initial post-treatment phase.

In three patients with Addison's disease treated with cortisone and desoxycorticosterone the metabolic effects were much more intense, and the catabolic process continued for ten days after discontinuing the progesterone.

The nitrogen loss averaged 2–4 gm. daily. Parallel losses of inorganic phosphorus and potassium were distinct and an enhanced creatinuria was observed in one patient. The increased urinary sodium and chloride excretion were also greater and more enduring, sodium losses again exceeding those of chloride. In a fourth Addisonian managed with salt alone progesterone did not affect sodium and chloride excretion indicating that the natriuresis and chloruresis were attributable to interference with the renal effects of salt retaining adrenal steroids.

The greater protein loss induced in patients with deficient adrenals suggests that this catabolic property of progesterone may be opposed by the adrenals, possibly by an anabolic secretion. The catabolic effects of progesterone and the interaction between it and adrenal steroids is important in the study of pregnancy, the menstrual cycle and the growth process itself.

Infrared Spectroscopy of Human Hemoglobins. DANIEL L. LARSON and JOSEPH MCLAUGHLIN, Washington, D. C. (Introduced by George A. Perera).

Infrared spectra of normal adult hemoglobin, fetal hemoglobin, sickle hemoglobin and hemoglobin C have been studied using a double beam recording infrared spectrophotometer. All samples were converted to carboxyhemoglobin before examination. Air dried hemoglobin films, cast from aqueous solution, showed almost complete loss of electrophoretic mobility. In order to avoid excessive infrared absorption due to the presence of water, hemoglobin samples were dried in vacuum from the frozen state and then dissolved in deuterium oxide or sodium deuteroxide. Hemoglobin solutions prepared in this manner showed essentially normal solubility and electrophoretic mobility.

Deuterated hemoglobin specimens were placed between silver chloride plates using platinum foil as a spacer and examined over a pH range up to 11.5. The various species of hemoglobin demonstrated differences in the rate of disappearance of the peak at 1545 cm^{-1} (6.47 microns) and the enlarging of the peak at 1440 cm^{-1} (6.93 microns). These rate changes appeared to be dependent upon the pH at which the examination was carried out. The significance of these variations in the infrared spectrum of the human hemoglobins will be discussed.

Urinary Excretion of Sodium and Potassium During the Pyrogenic Reaction. WILLOUGHBY LATHEM, New Haven, Conn. (Introduced by Paul B. Beeson).

Since serum and extracellular fluid electrolyte concentrations are usually maintained during fever, corrective renal adjustments apparently occur. Whether this compensation appears acutely when intrarenal vascular adjustments and hormonal mechanisms may be operative or only during prolonged pyrexia when extrarenal loss and internal shifts of water and electrolytes have occurred has not been determined.

The acute (three hours) effects of intravenous triple typhoid vaccine on urinary sodium and potassium excretion, glomerular filtration rate (GFR-inulin clearance) and renal plasma flow (RPF–PAH clearance) were determined in normal subjects. After a latent period RPF and GFR usually decreased slightly and sodium, potassium and urine output fell sharply. Since electrolyte and water excretion diminished independently of changes in GFR, augmented tubular reabsorption occurred. After 30–45 minutes GFR returned to control levels, urine output rose, and RPF increased markedly. Sodium excretion rose abruptly and exceeded control values significantly in most subjects (on the average by 60%). Potassium output continued to fall or returned toward control levels. Plasma sodium and potassium and venous hematocrit changed insignificantly.

Since similar results occurred in febrile and afebrile (aminopyrine premedication) subjects these acute changes are not attributable to fever or to extrarenal loss of electrolytes. The transient fall of both sodium and potassium excretion resembles the acute response to epinephrine/norepinephrine and suggests that sympathetic discharge may have been responsible. However, hormonal mechanisms do not appear to account for augmented sodium output during hyperemia. The mechanism of this altered tubular activity is obscure, but such factors as undetected internal water and electrolyte shifts or possibly intrarenal hyperemia alone may have contributed. Since urinary sodium loss usually does not occur during prolonged pyrexia later adjustments apparently abolish this natriuresis.

The Transfer of Generalized Cutaneous Hypersensitivity of the Delayed Tuberculin Type in Man by Means of the Constituents of Disrupted Leucocytes. H. SHERWOOD LAWRENCE,* New York, N. Y.

It is possible to induce, regularly, immunologically specific generalized cutaneous hypersensitivity of the delayed tuberculin type in animals and man by means of an injection of leucocytes obtained from sensitive donors.

In the studies outlined below, it has been found in the human, in contrast to animals, that leucocytes can be disrupted either by distilled water lysis or by repeated freezing and thawing and the resultant suspension of nucleoprotein sediment in cytoplasmic solution retains the capacity to transfer generalized delayed cutaneous hypersensitivity.

In 6 consecutive instances the constituents of distilled water lysed leucocytes and in 4 consecutive instances the constituents of repeatedly frozen and thawed leucocytes, obtained from the blood of donors sensitive to streptococcal M substance, when injected into M-negative recipients resulted in the development of generalized delayed cutaneous M substance hypersensitivity in each.

To substantiate this observation and to attempt identification of the particular cell component(s) involved in the mechanism of sensitization, the studies were repeated using tuberculin as the test material.

For 16 tuberculin negative recipients, suspensions of the constituents of repeatedly frozen and thawed leucocytes were prepared, on different occasions, from two tuber-

culin sensitive donors. Each of 8 alternate recipients received an injection of such untreated suspensions; of the remainder, 4 recipients received desoxyribonuclease treated suspensions and 4 recipients received ribonuclease treated suspensions. In 16 consecutive instances generalized delayed cutaneous tuberculin hypersensitivity developed in each negative recipient and the pattern of response appeared similar in the test and control groups.

The data demonstrate that in the human the transfer of generalized delayed cutaneous hypersensitivity can be accomplished regularly with a suspension of the constituents of disrupted leucocytes and suggest that the mechanism responsible for this effect does not appear to depend upon the polymerized desoxyribonucleic or ribonucleic acid components of the donor cell.

The Mechanism of the Osmotic Adjustment of the Body Cells. ALEXANDER LEAF,* JACQUES Y. CHATILLON, OLIVER WRONG and ELBERT P. TUTTLE, JR., Boston, Mass.

There has been renewed interest in the adjustment of the body cells to changes in extracellular fluid concentration. A solution to this problem was obtained in vivo by determining the volume of distribution of a large intravenous water load from the dilution of the serum sodium and total solutes.

In fifteen initial experiments, a water load was administered rapidly to dogs whose excretion of water was minimized by Pitressin. The average volume of distribution of the water load was $65.6 \pm 3.2\%$ and $63.6 \pm 2.3\%$ of body weight as calculated from changes in serum sodium and total solute concentrations, respectively. This measures net movement of water through the body.

To compare the volume of distribution of a tracer molecule of water which requires no net movement of water, simultaneous measurements of total body water content by D_2O were made in 10 additional experiments in dogs, either nephrectomized or with ureters ligated. The average total body water was $63.0 \pm 1.1\%$ of body weight. The average volume of distribution of the water load was $61.2 \pm 1.5\%$ and $67.6 \pm 1.8\%$ of body weight as calculated from changes in serum sodium and total solute concentrations, respectively.

The close agreement between total body water and the volume of distribution of a large water load indicates that in vivo the osmotic adjustment of the overwhelming majority of body cells is accomplished by passive entry of water into the cells. Further conclusions from the data are: 1) The water load distributes itself so that the ratio of intracellular to extracellular water is unchanged. 2) Active transport of water to prevent changes in intracellular osmotic activity does not occur. 3) Osmotic equilibrium during dilution is not attained by osmotic inactivation of intracellular solutes or by their extrusion from the cells. 4) Significant release of sodium from bone or from within the cells in response to acute dilution does not occur.

A Blood-born Vasoconstrictor Factor in Hypertensive and in Normotensive Subjects. RICHARD E. LEE, New York, N. Y. (Introduced by H. D. Lauson).

With the use of a new method of bio-assay, extremely low levels of vasoconstrictor activity can be detected in the body fluids of man and animals. The vasoconstrictor factor (VCF) present in such liquids as tissue washes, plasma, urine, and sera can be expressed in standardized physiological equivalents of epinephrine as gamma/cc.

The plasma level of VCF in hypertension and in normotensive patients and subjects is equal to 0.0001–0.001 gamma/cc. epinephrine. It is relatively constant in each individual providing that the blood pressure is stable. Transient hypertension and severe hypotension are associated with highly significant increases in plasma VCF. In certain normotensive subjects, emotional trauma is accompanied by comparable changes in plasma VCF without blood pressure change, of note. Preliminary evidence is given associating high plasma levels of VCF with the "false positive" Regitine test.

The possible significance of this new factor is discussed in relation to human peripheral vascular physiology.

The Use of Tritium- and Radiocarbon-Labeled Precursors to Study the Metabolism of Steroid Hormones in Man. GEORGE V. LEROY, DELBERT BERGENSTAL, R. GORDON GOULD, HAROLD WERBIN and GEORGE T. OKITA, Chicago, Ill. (Introduced by Clayton Loosli).

The role of acetate and diet cholesterol as precursors of adrenal corticosteroids was investigated by means of a double-isotope labeling technic, using three patients who underwent bilateral adrenalectomy and oophorectomy. For several days before surgery ACTH was injected; and tritiated cholesterol was fed daily in sufficient amounts to provide a nearly constant value for the specific activity of the plasma-free-tritium-cholesterol.

During the preoperative period, pooled urine samples were collected and the 17-ketosteroid fractions isolated were found to contain T-labeled ketosteroids. Approximately 1½–2 hours before the adrenals were removed an intravenous dose of 1-C^{14}-acetate was given to "flash label" the cholesterol currently being synthesized by the patient. The urine collected during the first few days postoperatively contained, in addition to the T-labeled steroids, C^{14}-labeled-17-ketosteroid fractions presumably formed during the two hours before removal of the adrenals and ovaries. In addition to the urine studies, determinations were made of the specific activities of tritium—and carbon 14—labeled adrenal cholesterol, and of the cholesterol in blood and other tissues obtained at surgery. The relationship between the specific activities of plasma-free-T-cholesterol, adrenal-free-T-cholesterol, and T-17-ketosteroid indicated that diet cholesterol was a precursor of urinary ketosteroid. The specific activity of adrenal-free-C^{14}-cholesterol, in every case, was several times greater than the specific activity of plasma-free-C^{14}-cholesterol, demonstrating that the ACTH-stimulated gland synthesized cholesterol rapidly from the 1-C^{14}-acetate. Because of the fact that adrenal-free-C^{14}-cholesterol

reached such high values so rapidly it is not possible to say whether the acetate went directly into steroid hormones, or whether the C^{14}-ketosteroids were formed from the newly synthesized C^{14}-cholesterol. The specific activity of the urinary C^{14}-17-ketosteroid was invariably less than that of the adrenal-free-C^{14}-cholesterol. To accomplish these studies, methods were developed using a liquid scintillator for the determination of tritium and C^{14} in the same sample of plasma or tissue cholesterol, and in pure fractions of urinary steroid.

The Venous Pressure-Volume Curve of the Human Leg Measured in Vivo. JULIUS LITTER and JAMES E. WOOD, Boston, Mass. (Introduced by James M. Faulkner).

The purpose of this study was to develop a method for measuring venous pressure-volume curves of the leg which would be physiologically valid. Such curves should represent the effects of known venous pressures on venous volume.

A constant reference point or baseline value of venous volume at an effective venous pressure slightly greater than zero in all the veins was obtained when the horizontal leg was placed in a water-filled plethysmograph and the water pressure on the leg was greater than the natural local venous pressure. The actual venous pressure in the leg was adjusted to equal an arbitrary pressure in a venous congesting cuff on the thigh by "titrating" the water pressure on the leg and recording volume changes. Then the congesting cuff pressure was increased 30 mm. Hg by increments of 5 mm. Hg and the corresponding increases in leg volume were recorded. Experimental evidence will be presented indicating that these pressure-volume curves were produced by increasing the effective pressure in all the leg veins from slightly greater than zero to 30 mm. Hg by increments of 5 mm. Hg.

The average total increase in venous volume was 4.75 cc. per 100 cc. of leg in healthy young men. Other studies have shown that the total vascular volume varies between 1.5 cc. and 1.0 cc. per hundred cc. of leg over a wide range of external pressures, the venous volume remaining constant at an effective venous pressure slightly greater than zero. Accordingly, the volume and distribution of blood in the human leg are mainly functions of the effective venous pressure.

The curves are not affected by hemodynamic changes and are determined by only one variable, the resistance of the veins to stretch. Therefore they are useful for the study of venous tone. This will be illustrated by the effects of epinephrine and norepinephrine.

The Response of Bone, Connective Tissue and Muscle to Sudden Changes in Extracellular Fluid Composition and Volume. M. F. LEVITT, L. B. TURNER, A. Y. SWEET and D. GRIBETZ, New York, N. Y. (Introduced by Milton Mendlowitz).

Electrolyte and water analyses of cortical (marrow-free) bone, tendon, and muscle were performed in normal rats and in rats rendered acidotic, alkalotic, and salt-depleted during a four-hour interval. These states were produced by intraperitoneal administration of ammonium chloride, sodium bicarbonate and distilled water, respectively, in amounts calculated to augment total extracellular chloride or sodium or to deplete total extracellular salt by approximately 20%.

Chloride acidosis induced: 1. A reduction in extracellular phase of muscle of 15% as reflected in a reduced sodium and chloride space of muscle, together with a proportionately smaller increment in intracellular phase. 2. A fall of 0.025 meq., 0.015 meq. and 0.008 meq. of sodium/gm. of dry bone, tendon and muscle respectively, beyond that attributable to changes in extracellular fluid.

Sodium alkalosis induced: 1. A 10% expansion of the extracellular phase of muscle as reflected in the increased sodium and chloride space of muscle, together with a reduction in intracellular phase.

Salt depletion induced: 1. A predicted 20% reduction in the extracellular phase of muscle and a simultaneous expansion of the intracellular phase. 2. A fall of .020 meq. of sodium/gr. of dry bone and .015 meq. of chloride/gm. of dry tendon beyond that attributable to changes in extracellular fluid.

Approximately 90% of cortical bone sodium and a considerable fraction of connective tissue chloride and sodium do not appear to be contained in the extracellular fluid. Instead, such stores of salt are probably confined by the tissue matrix and may, in part, be rapidly released to maintain extracellular fluid composition and volume. Acute alterations in extracellular fluid pH appear to produce immediate changes in the volume of the extracellular phase of muscle, with reciprocal changes in intracellular volume.

The Relationship Between Changes in Thyroidal I^{131} Uptake and Serum Protein-Bound Iodine Following Thyrotropin. RICHARD P. LEVY, LUTHER W. KELLY, JR. and WILLIAM MCK. JEFFERIES, Cleveland, O. (Introduced by Max Miller).

A single intramuscular injection of thyrotropin (TSH) in normal persons produces a significant rise in the thyroidal uptake of I^{131} and in the serum protein-bound iodine (PBI) within 24 hours. The former reflects an increased rate of iodide trapping and the latter an increased release of thyroid hormone. A question arises regarding the relationship between these two effects of TSH stimulation, i.e. whether one might be dependent upon the other.

When thyroid extract is administered to normal persons the uptake of I^{131} is depressed, reflecting decreased endogenous TSH effect, but the response to injected TSH is still brisk. The PBI response, however, is not so consistent. After ingestion of comparatively large doses of thyroid over prolonged periods, no rise in PBI follows a single TSH injection. Presumably prolonged inhibition of endogenous TSH results in an atrophic thyroid gland which is not able to produce a significant rise in PBI in 24 hours, but which can still respond promptly with an increased trapping of iodide.

Studies of cases of hyperthyroidism following subtotal thyroidectomy or I^{131} therapy have also produced evidence that these two effects are not necessarily interre-

lated, even when the thyroid tissue is under strong endogenous TSH stimulation. One patient had a normal increase in I^{131} uptake in response to TSH, but no rise in PBI. In this instance the residual tissue was apparently forming and releasing thyroid hormone at a maximum rate, but the iodide uptake was not maximal. In two other patients TSH injection produced no rise in uptake, but a significant increase in PBI occurred. Here the uptake mechanism was apparently operating at a maximum rate, but that controlling the release of hormone was not.

Hence it appears that these two effects of TSH are not necessarily related, although under normal circumstances they usually occur together.

Studies of Inactivation and Formation of Antidiuretic Substance by Hypophysectomized Rats. CHARLES W. LLOYD,* ERICH H. LOEWY and SOPHIE PIEROG, Syracuse, N. Y.

Normal and hypophysectomized young adult male Sprague-Dawley rats were pair fed a diet high in calories and protein which maintained good growth rates in both groups. The hypophysectomized animals had greater fluid intake and urine volume than the normal animals. Daily administration of Pitressin Tannate in oil (1 milliunit/gram) caused a greater decrease in fluid intake and output in the hypophysectomized rats and this was maintained throughout the administration of Pitressin. The normal animals had a decrease in urine volume lasting for the first few days of Pitressin Tannate administration but had no change in fluid intake. Hyponatremia and hypochloremia developed in the normal but not in the hypophysectomized animals. This difference was probably due to greater dilution in the normals. The livers of the pair-fed hypophysectomized animals had normal ability to inactivate Pitressin *in vitro*. Daily priming with Pitressin did not alter this ability in either group. Since hypophysectomized animals which became cachectic on a diet of ordinary laboratory pellets had a marked decrease in hepatic ability to inactivate Pitressin, it appears that this defect is primarily the result of nutritional rather than endocrine factors. Serum of recently operated animals had no activity. Serum of many animals hypophysectomized for 30 days contained essentially the same amount of antidiuretic activity as was found in normal serum. This suggested that reorganization had taken place, permitting release of ADH from the hypothalamus. Measurement of hypothalamic antidiuretic activity demonstrated little activity immediately after operation. The hypothalamic antidiuretic activity of animals operated one month was higher than in normal animals. This activity was concentrated in the area containing the median eminence. It was decreased by dehydration and increased by hydration. Pentobarbital appeared to increase the antidiuretic activity of the hypothalamus.

Melanuria. GEORGE D. LUDWIG, Philadelphia, Pa. (Introduced by Francis C. Wood).

Melanuria is usually associated with metastatic melanoma. However, we have observed it in a variety of diseases, and suggest possible alterations of pigment metabolism that may have caused it. Melanin, or its precursor, was demonstrated spectrophotometrically, by five specific chemical tests, and by isolation of the pigment, in the following conditions: chronic liver disease with hyperpigmentation, peri-cholecystic abscess, hemochromatosis, argyria, Addison's disease, and metastatic melanoma.

Melanin is a polymer derived from tyrosine by a series of reactions involving the enzyme, tyrosinase, and autooxidative steps. In man, the metabolic fate of tyrosine includes urinary excretion, enzymatic degradation in the liver, and conversion of small amounts to epinephrine, thyroxine, and melanin.

In chronic liver disease, the normal mechanism for metabolizing tyrosine may be depressed as evidenced by tyrosinemia and tyrosinuria. Increased supply of substrate to tyrosinase may be an important factor leading to hyperpigmentation and melanuria occasionally seen in these patients, although other factors are involved.

Local tissue necrosis occurred with the peri-cholecystic abscess. Pigment production may have resulted from increasing substrate by liberation of phenolic groups of proteins, by pH changes, or from decrease of sulfhydryl groups, which have been proposed as normal inhibitors of tyrosinase.

Increased dermal melanin deposition commonly found in hemochromatosis and argyria, and the melanuria may result from sulfhydryl binding or from direct acceleration of the enzymatic reaction by metal ions. Both phenomena have been demonstrated *in vitro*.

Melanuria was found in chronic Addison's disease with hyper-pigmentation, but not in acute surgically induced hypoadrenalism. The mechanism of increased pigment production in Addison's disease is unknown, but the recent proposal that the adrenal regulates tissue sulfhydryl groups is in best agreement with present experimental data.

Spectral Phonocardiography: Studies of Heart Sounds and Murmurs with a Modified Bell Sound Spectrograph. VICTOR A. MCKUSICK, SAMUEL A. TALBOT and GEORGE N. WEBB, Baltimore, Md. (Introduced by E. Cowles Andrus).

The Bell sound spectrograph was adapted for phonocardiography by these modifications: 1) Analyzing passband filter widths of 25–35 c.p.s. provide optimum simultaneous resolution in time and frequency. 2) Parallel recording of EKG, etc., has been instrumented. 3) A logarithmic display of frequency spectrum has a more realistic appearance and advantageously "spread out" the lower frequency range where greater energy content is concentrated.

Spectral phonocardiograms incorporate all three parameters of heart sounds and murmurs in a single, detailed, analytical display.

Time is displayed on the abscissa, frequency spectrum on the ordinate; intensity range is represented by degree of blackness of particular portions of the records (on electrically sensitive paper). Precise frequency × intensity analyses at specific points in time are also possible.

Spectral phonocardiograms resemble the mental images of cardiovascular sounds. Their interpretation is easy for the experienced stethoscopist. Their value in teaching and for permanent clinical records is great. Artefacts due to ambient vibrations or to electrical interference can be recognized as such and disregarded.

Quantitative analyses of frequency content are presented. Some murmurs and altered heart sounds have frequency components at 1500 c.p.s. or even higher. Higher frequency components are important in stethoscopy because of the Fletcher-Munson characteristics of the ear.

The phonetics of the heart, i.e., the physical basis of many adjectives used in describing the quality of these sounds, is elucidated: e.g., "cooing" diastolic murmurs are characterized by a clear-cut fundamental with harmonics and a distinctive curvature. Fundamental-harmonic systems have also been found in musical systolic murmurs. (The physical basis for the terms applied to percussion notes, breath sounds, voice sounds, and rales has been investigated.)

We seek information which is otherwise unobtainable clinically and which may be useful in the differentiation of sounds of identical stethoscopic character but different etiologic, prognostic, and functional significance.

Clinical Application of a Sensitive Method for Detecting Antihormones in Patients Receiving Pituitary FSH. WILLIAM O. MADDOCK, ICHIRO TOKUYAMA, ROBERT B. LEACH and C. ALVIN PAULSEN, Detroit, Mich. (Introduced by Gordon B. Myers).

Conventional methods for detecting antihormone formation in patients receiving pituitary FSH are based upon the ability of the patients' plasma to prevent the effects of the FSH administered to immature female rats or mice. These methods are so insensitive that clinical evidence of refractoriness usually appears before antihormone activity can be demonstrated in the plasma, and antihormones usually are no longer detectable one to three months after stopping treatment. The antihormones which develop in patients receiving pituitary FSH preparations are capable of blocking the action of rat pituitary FSH. This suggested a sensitive antihormone assay method, based on the fact that the presumably minute amounts of endogenous FSH circulating in the immature female rat are necessary for chorionic gonadotrophin (CG) to stimulate ovarian estrogen secretion. Thus, to prevent the action of CG (increased estrogen secretion as evidenced by enlarged uteri) only enough antihormone need be present to block the assay rat's endogenous circulating gonadotrophin. This method was compared with the conventional assay for detecting antihormones in six patients who received hog pituitary FSH for 2 months or longer. The "CG method" was 10 to 20 times more sensitive than the conventional assay and permitted detection of antihormones at the onset of clinical refractoriness and for as long as 12 months after stopping FSH treatment.

In keeping with the interpretation that the antihormones block CG action indirectly, by neutralizing the female rats' endogenous gonadotrophin, the antihormones did not block the direct, Leydig cell-stimulating action of CG in *male* rats which does not require the presence of pituitary gonadotrophin. In two men having high antihormone levels as the result of hog FSH therapy, CG therapy produced a prompt response as judged by increased urinary estrogens and 17-ketosteroids and symptoms of enhanced androgen production.

Succinic Dehydrogenase Activity in Tuberculous Animals. S. P. MARTIN,* S. N. CHAUDHURI and C. D. COOPER, Durham, N. C.

In studies on the metabolic response of guinea pigs to tuberculosis it was noted that the non-caseating tissues, such as kidney, had a low succinic dehydrogenase activity. This loss of activity was also noted in animals suffering from hemorrhagic shock. The activity could be restored by addition of a factor present in normal kidney cortex.

Chromatographic studies of normal tissues on paper and ion exchange column revealed that a nucleotide was responsible for activation. This nucleotide contains adenine, ribose, two phosphate groups and three alpha amino groups. There is a free sulfhydryl after treatment with cyanide and Toennes reagent. The factor is thermolabile. Unchromatographed adenine diphosphate contains an impurity that will serve as a basis for the nucleotide when tissues are incubated with glutathione, cysteine and cystine. Dinitrophenol blocks the restorative capacity of the nucleotide. This effect cannot be produced by the known nucleotides including coenzyme A.

These data would indicate that animals in shock or severely infected with tuberculosis have a low succinic dehydrogenase activity. This lowered activity appears to be related to a lowering of a tissue nucleotide. The nucleotide may operate in one of three ways: It may remove oxalacetate from the system. It may be related to the Slater factor. Also, this compound might serve as as a link in oxidative phosphorylation. The latter speculation has the greatest experimental proof. The loss of this material by the cell may play a role in the pathogenesis of this disease.

Laboratory and Clinical Studies of a Modified Hemolytic Test for Tuberculosis. C. MIDDLEBROOK,* H. OZERENGIN and R. OESTREICHER, Denver, Colo.

The unsatisfactory results obtained by many investigators on application of the originally described hemolytic test for the diagnosis of tuberculosis led us to investigate the causes of false positive reactions in this test. The following causes have been discovered and eliminated:

1. A serologic cross-reaction exists between a component of old tuberculin and Staphylococci: a special method of fractionation of tuberculin has permitted removal of this component from the specific red-cell-sensitizing antigen.

2. A labile red-cell-lyzing substance which appears in some sera from non-tuberculous individuals can be inactivated by treating the diluted serum with calcium ions

at 37°C. for two hours before submitting it to the hemolytic test.

3. A serologic cross-reaction between tuberculin and histoplasmin, causing the sera of certain patients suffering from histoplasmosis to give a false positive reaction in the hemolytic test for tuberculosis, can be eliminated by preliminary absorption of such sera with small amounts of histoplasmin—a procedure which has no effect on the titres of sera from tuberculous individuals.

Application of the hemolytic test, modified as a result of identification of the above causes of false positive reactions, in the study of over 1,300 sera from non-tuberculous groups of human beings gave less than 3% false positive reactions. The same modified test was applied to sera from a large number of tuberculous individuals. Approximately 75% of untreated cases gave "doubtful" or "positive reactions." It has also been observed that chemotherapy with isoniazid and streptomycin has a "provocative" effect on humoral antibodies as measured by the hemolytic test: more than half of the untreated cases which gave "false negative" reactions before therapy became serologically positive in the hemolytic test within a few months after initiation of such therapy.

It is concluded that the modified hemolytic test has proven useful as a diagnostic test for tuberculosis.

Total Liver Phosphorus: Its Relation to Hepatic Glycogen Deposition. NANCY NICHOLS, FREDY E. SCHERTENLEIB and EMERY C. MILLER, Boston, Mass. (Introduced by Alexander Marble).

Costisone, administered for 18 days, caused a 100% increase in liver weight in both normal and alloxan-diabetic rabbits. The increase in liver size was due entirely to the intracellular deposition of glycogen and water. The percentage of intracellular water in the whole liver was unchanged and its potassium concentration remained constant.

In contrast to the increase in concentration of acid-soluble phosphate associated with hepatic glycogenesis, the concentration of total phosphate fell from 245 mM/Kg intracellular water in glycogen-free livers to 126 in livers in which glycogen constituted 50% of the dry solids. A constant P: N ratio of 3.73 (mM phosphate: Gms nitrogen) was found in all livers. In the intracellular water of glycogen-free liver, total phosphate was so high that more than half must have been bound, possibly to protein, if the criteria of osmotic equality and electroneutrality are to be satisfied. It is postulated that this excess phosphate, present in liver but not in muscle, forms an intrahepatic reservoir of phosphate ions which, as glycogen is deposited, can readily change their state, entering the pool of acid-soluble phosphate and supplying anion equivalence and osmolar force to the potassium and water which are deposited with glycogen.

Furthermore, since it appears from these data that no extrahepatic phosphate enters the liver during glycogenesis, it is concluded that the fall in serum phosphate associated with the intravenous administration of glucose is related exclusively to the peripheral utilization of carbohydrate.

The Role of Venomotor Activity in Circulatory Adjustments. ERNEST B. PAGE, HERBERT O. SIEKER, JOHN B. HICKAM * and WILLIAM W. PRYOR, Durham, N. C.

Venomotor activity is involved in normal and abnormal circulatory dynamics, but its magnitude and importance have been difficult to assess. The present study is concerned with methods of measuring venous reactions, the pattern of normal venomotor responses, and venomotor abnormalities in cardiovascular disease.

Changes in central venous pressure, reflecting venomotor activity in the body as a whole, were compared with pressure changes in a superficial vein segment isolated by external pressure (Duggan). In sixteen normal subjects observations have been made on responses to the Valsalva maneuver, cold, exercise, postural change, and hyperventilation. After the Valsalva maneuver, which produced the greatest and most consistent response, there was a mean rise of 16 mm Hg in the vein segment and 4 cm H_2O in central venous pressure. Tilting and hyperventilation also caused pressure rises in both regions, though less marked. With cold and exercise the superficial segment constricted well, but central venous pressure changes were inconsistent. Since the two regions do not always change together, it appears that different stimuli can invoke different patterns of venous constriction. This venomotor activity has obvious importance in controlling the distribution of blood volume throughout the body.

Four patients with diabetic, idiopathic, and post-sympathectomy hypotension showed markedly impaired or absent venous responses to the standard stimuli. Stimulation of a sensitive carotid sinus, or coughing, in a patient with cough syncope produced simultaneous falls in central venous and arterial pressures without change in heart rate. It is concluded that impairment of venomotor activity, with consequent decrease in venous return to the heart, plays a significant part in the disability of such patients.

Pituitary Somatotropin as a Growth for Human Mammary Carcinoma. OLOF H. PEARSON,* BRONSON RAY, CHARLES D. WEST, CHARLES C. HARROLD, JOHN P. MACLEAN and MIN CHIU LI, New York, N. Y.

Oophorectomy and adrenalectomy will induce objective remissions in patients with estrogen dependent mammary cancer. Olivecrona and Luft have reported improvement from hypophysectomy in patients with metastatic mammary carcinoma. The question arises whether hypophysectomy induces improvement simply by suppression of gonadal and adrenal function, or whether there is in addition a pituitary hormone which is important in maintaining the growth of breast cancer.

Two women with active metastatic osteolytic mammary carcinoma, who had previously undergone oophorectomy and adrenalectomy with transient improvement, have undergone hypophysectomy. Both patients have obtained objective remissions as evidenced by improvement of severe myelophthisic anemia, cessation of osteolysis, and calcification of osteolytic lesions in one patient. The remissions continue 7 and 3 months after hypophysectomy.

Beef pituitary somatotropin was administered intramuscularly in doses of 100 milligrams daily for 18 days to one of these patients three months after hypophysectomy, while on a metabolic balance regime. During growth hormone administration, there was a steady rise in urinary calcium excretion from 165 milligrams to 408 milligrams per day. There was a concomitant rise in urinary phosphorus and in fecal calcium and phosphorus excretion. Nitrogen and potassium balances became slightly negative during growth hormone administration. When somatotropin was stopped, urinary calcium and phosphorus excretion promptly returned to control levels. These data indicate that growth hormone induced osteolysis presumably due to stimulation of growth of osteolytic tumor.

These observations suggest that pituitary somatotropin is an important growth factor for human mammary carcinoma.

Studies on a Salt Retaining Factor Prepared from Human Urine. MAURICE M. PECHET, LEROY E. DUNCAN, JR., GRANT W. LIDDLE and FREDERIC C. BARTTER,* Bethesda, Md.

A salt retaining factor was prepared from human urine by the following method. The urine was acidified to pH 1 with hydrochloric acid, extracted exhaustively with ether and methylene chloride, and the methylene chloride extract washed with alkali and water. The carefully dried extract was subjected to silica gel column chromatography. Fifteen fractions were eluted. Bioassays for sodium retention and potassium excretion in adrenalectomized dogs indicated that the active material was eluted with methylene chloride-methanol mixture. This fraction was subjected to paper partition chromatography in the Pechet system. The migration rate of the active material was different from those of cortisone and hydrocortisone. The material was characterized by ultraviolet absorption, reaction with alkaline silver nitrate and blue tetrazolium. Of the various urines studied the material obtained from urine of patients with cirrhosis and ascites showed the highest degree of increased activity over that from normal subjects.

Evaluation of the salt retaining activity of this factor when administered to human subjects will be discussed.

Abnormalities of Carbohydrate Metabolism of Red Cells in Hereditary Spherocytosis. THOMAS A. J. PRANKERD, KURT I. ALTMAN and LAWRENCE E. YOUNG,* Rochester, N. Y.

Since many hereditary diseases are known to be due to defects in intermediary metabolism, experiments were designed to determine if such an abnormality could be detected in red cells from patients with hereditary spherocytosis (HS).

Normal and pathological human erythrocytes were incubated with $NaH_2P^{32}O_4$ at 37° C with constant bubbling of 95% O_2 and 5% CO_2 to insure mixing and constant pH. Phosphate intermediates involved in glycolysis were separated unequivocally from trichloracetic acid extracts of "intracellular" and stromal fractions of these cells by paper chromatography.

Normal erythrocytes take up P^{32}-phosphate from extracellular medium at reproducible rates, and the distribution of radioactivity within the cells follows a constant pattern. 2,3-diphosphoglyceric acid (DPG) bears a precursor relationship to adenosine triphosphate (ATP) and both fractions show greater activity than inorganic phosphate. Stromal fractions show moderate activity of both 2,3-DPG and ATP with magnitudes and time courses different from those demonstrated intracellularly. Incubation with adenosine has only slight effect on relative specific activities (RSA's) during the first six hours.

Cells from HS patients, before and after splenectomy, take up P^{32}-phosphate at the same rate as normal cells, but relationships among intracellular fractions differ markedly, the RSA of inorganic phosphate exceeding that of either 2,3-DPG or ATP. Stromal fractions have low activities. Cells from certain of these cases incubated with adenosine show intracellular relationships restored closely to normal, but stromal activities are not affected. Phosphate metabolism in cells from other HS cases is not altered significantly by adenosine. This and other biochemical findings, together with recent hematological observations, indicate that the group of cases in this clinic regarded as examples of HS may not be homogeneous.

Results such as these suggest that "inborn errors" of erythrocyte metabolism may be partly responsible for the hematological features of hereditary spherocytosis and other congenital hemolytic disorders.

A Study of the Use of Rb^{86} as a Tracer for Measurement of Rb^{86} and K^{42} Space and Mass in Intact Man with and without Congestive Heart Failure. C. THORPE RAY,* SAM THREEFOOT and G. E. BURCH,* New Orleans, La.

Since rubidium enters potassium metabolic pool, attempt was made to estimate Rb^{86} space and potassium space and mass, utilizing radiorubidium. Rb^{86} (half-life 19.5 days) provided information about potassium metabolism for prolonged periods (70 days) unobtainable with K^{42}. One control and two subjects with heart failure were studied under controlled metabolic conditions. Intracellular (erythrocytes) and extracellular (plasma) compartments were studied simultaneously.

Estimation of space and mass by isotope dilution assumes equilibrium of distribution of isotope in all compartments. Absolute equilibrium of distribution was not achieved since (1) erythrocyte Rb^{86} increased 5–8 days after injection, whereas plasma Rb^{86} decreased, (2) plasma Rb^{86} declined independently of erythrocytes and (3) both plasma and erythrocyte Rb^{86} declined more rapidly than could be explained by excretion from body. This would likewise apply to K^{42}.

Although estimated potassium space and mass utilizing erythrocyte space and mass equivalents varied less than estimations based on plasma determinations, enough temporal variation existed to indicate unreliability of calculations based on single observations. Space and mass

calculated from plasma values increased progressively, reflecting more rapid excretion from plasma than from cellular compartments. Increased potassium intake caused more rapid Rb^{86} loss from plasma than erythrocytes, marked increases of space and mass utilizing plasma space and mass equivalents, but only slight increases using erythrocyte space and mass equivalents. Therefore, in estimating space and mass of substances, the *major* compartment should always be sampled, i.e., for primarily intracellular substances (Rb, K), the intracellular compartment, and for primarily extracellular substances (Na, Cl), the extracellular compartment.

Utilizing average erythrocyte values Rb^{86} space was 54.6, 49.1 and 43.5% body weight and potassium mass was 136.7, 104.3 and 91.0 grams or 0.196, 0.186 and 0.164% body weight for control, patient with moderate CHF and severe CHF respectively. These values increased slightly on higher potassium intake.

The Relation of Urinary Ammonia to Urine pH. FLOYD C. RECTOR, JR., ALBERT D. ROBERTS, JR. and JOHN COPENHAVER, Dallas, Tex. (Introduced by Ben Friedman).

The rate of hydrogen ion secretion has been considered to be the factor regulating ammonia excretion. To elucidate this relationship, H^+ formation was selectively blocked by giving rats carbonic anhydrase inhibitor (Diamox) for 14 days. Urine was analyzed for pH, titratable acid, and ammonia. Serum and muscle electrolyte composition was determined. Activities of glutaminase and carbonic anhydrase were measured in kidney homogenates.

Diamox resulted in a sustained rise in urine pH and fall in titratable acid excretion. Despite the alkaline urine, ammonia excretion rose from $1.71 \pm .20$ meq. daily in controls to $2.27 \pm .25$ meq. daily with Diamox alone and $2.32 \pm .28$ meq. daily with Diamox + KCl. Paralleling the increased ammonia excretion renal glutaminase activity rose from 788 ± 39 units (M NH_3/100 mgm. dry kidney/hour) in controls to 2246 ± 487 units with Diamox alone and 1910 ± 193 units with Diamox + KCl. Intracellular K^+ decreased from 51.4 ± 1.2 meq./100 gms. fat-free dry muscle (FFDM) in controls to 37.9 ± 6.1 meq./100 gm. FFDM in experimental animals.

Potassium deficiency cannot be responsible for accelerated ammonia excretion in rats given Diamox, since correction of the potassium deficit prevented neither increased ammonia excretion nor increased glutaminase activity. The possibility of intermittent Diamox inhibition, with consequent post-inhibitory rebound phenomena, was eliminated by giving the inhibitor at 4–8 hour intervals. Following a single injection of Diamox there was still approximately 25% inhibition of carbonic anhydrase activity at the end of 8 hours. Therefore, significant inhibition was maintained at all times.

Increased urinary ammonia in an alkaline urine indicated that ammonia excretion was at least partially independent of urine pH. It is suggested that the increased ammonia excretion was the consequence of increased renal glutaminase activity, which may have been related to changes in intratubular acid-base equilibrium.

Mitral Stenosis: The Effects of Digoxin with Exercise. E. A. S. REID, A. B. JUDD, D. J. MACINTOSH, I. G. MILNE, T. R. HALE and M. MCLAUGHLIN, Montreal, Canada. (Introduced by J. S. L. Browne).

Although investigators have reported upon the effects of Digoxin on the function of the human heart modified by mitral stenosis while the patient was at rest, the effect of this drug during exercise appears to have been neglected. The present paper is a preliminary communication on this apparently unexplored problem.

Eleven patients with mitral stenosis were studied. Cardiac catheterization was performed and pressures and outputs determined. Exercise by leg flexion was carried out for five minutes and the data recorded. One milligram of Digoxin was administered intravenously. Studies were repeated after one hour.

Four patients, compensated and with normal rhythm, showed minor changes only, following digitalization.

Three patients, decompensated and with auricular fibrillation, showed improvement at rest in rate, output and diastolic pressures. With exercise there was little improvement over the control period. Diastolic pressures rose to a less degree.

Three patients, decompensated but with normal rhythm, showed the most improvement. The outputs were increased at rest and with exercise following Digoxin. At rest diastolic pressures fell but systolic pressures were unchanged. With exercise, there was a marked rise in systolic pressure during the control period but following Digoxin there was no rise at all.

These studies suggest that Digoxin has an effect, not only on the failing ventricle but also on the failing auricle when this auricle has a normal rhythm. We have shown that Digoxin has markedly improved the function of exercising "mitral stenotics" in failure when sinus rhythm is present, and that this improvement is absent when the auricle is fibrillating. In decompensated mitral stenosis after exercise, we conclude that Digoxin has a greater beneficial effect when normal rhythm is present than when the auricles are fibrillating.

Factors Influencing the Growth of Megaloblasts In Vitro: Effect of Vitamin B-12, Folinic Acid (CVF), A-Methopterin and Colchicine. EDWARD H. REISNER, JR. and HAROLD T. SWAN, New York, N. Y. (Introduced by L. Emmett Holt, Jr.).

Others have reported that megaloblasts grown in suspension cultures in PA serum showed normoblastic "ripening" with the addition of folic acid, but not with B-12 unless the latter was accompanied by intrinsic factor contained in normal gastric juice. We found that using their technique (a modified Osgood culture) when grown in suspension in PA serum for 48 hours megaloblastic marrows from PA patients showed a mean increase of nucleated red cells per mm^3 of 308; 561 with added B-12; 282 with intrinsic factor; 490 with B-12 plus intrinsic

factor, and 1028 with CVF. Only the latter change was statistically significant ($p < .05$). Maturation of cultures was accompanied by disappearance of megaloblasts and increase of normoblasts, more marked in the presence of B-12 or CVF. In contrast, when grown on chick plasma clots or glass surfaces B-12 alone appeared to be strongly stimulatory and to restore normoblastic hematopoiesis. (CVF was not tested by the latter methods.)

The increase of cells in megaloblastic marrow suspension cultures could be blocked with colchicine indicating it was due to mitosis. Normoblastic marrows showed no greater increase of nucleated red cells after 48 hours when CVF was added than in the control. A-Methopterin caused a decrease in cells which was completely reversed by CVF but not by folic acid.

Failure of suspension marrow cultures to be stimulated markedly by B-12 in contrast to cultures on clots and glass surfaces is surprising. Possibly, the minute quantities of B-12 present in relapsed PA serum are inadequate to support the more vigorous growth of the latter cultures but suffice for the limited mitotic activity in suspension. Since both B-12 and CVF must be present in minimal amount for mitosis, under such circumstances the amount of CVF present in the suspension could become the major factor influencing growth.

These studies fail to support the hypothesis that B-12 must be "activated" by intrinsic factor to function in hematopoiesis.

Studies in Endothelial Capillary Permeability: II. Congestive Failure. ALEXANDER P. REMENCHIK and ROBERT M. KARK,* Chicago, Ill.

Reliable methods for simultaneous determinations of red cell mass (space) and human serum albumin space were developed. These were applied to measurement of the spaces in patients with heart disease, before and after successful therapy of congestive failure. Red cell mass measures intravascular space. In congestive failure it is "normal" and does not change following successful therapy. Albumin space measures intravascular space *plus* an unknown portion of the extravascular compartment. The albumin space is increased in congestive failure and this implies that endothelial permeability to albumin is also increased, since albumin space shrinks to "normal" after therapy. Using data from others, together with our own observations, an hypothesis for the pathogenesis of congestive failure is presented and the physical findings in this condition are explained. In patients with heart disease, the decrease in myocardial efficiency produces a change in permeability of the capillary endothelium; the development of this change needs study. Transfer of protein to the extravascular compartment causes a fall in the quantity of intravascular protein and a transient decrease in intravascular volume. Homeostatic mechanisms for maintenance of blood volume are activated, among which is retention of sodium and water. These, with protein, enter the extravascular compartment. In addition, there occurs a reflex spasm of the venous "off-flow system" which leads to increased peripheral resistance and distention of the large veins. The above mechanisms account for the cold, cyanotic extremities, the distended liver, the distended veins, and the raised venous pressure seen in congestive failure. Peripheral space may be relaxed by injection of TEAC which produces a fall in venous pressure and loss of venous distention in patients with congestive failure.

Biochemical Sequence of Events After Insulin Administration to Diabetic Rats. ALBERT E. RENOLD and A. BAIRD HASTINGS, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

Whereas effects of insulin added *in vitro* to skeletal muscle preparations can be demonstrated with ease, effects of insulin added *in vitro* to hepatic tissue are most difficult to obtain. Indeed, the only clear-cut effect of insulin added *in vitro* to isolated liver tissue concerns the incorporation of acetate into fat, while attempts to demonstrate an insulin action on glycogen deposition, glucose utilization or glucose oxidation to CO_2, have been negative. This is particularly puzzling since marked differences between normal and diabetic liver incubated *in vitro* have been shown. In this study an attempt has been made to define the biochemical sequence of events during the reversion of diabetic liver metabolism towards normal by insulin administration.

Glucagon-free insulin was administered to diabetic rats in doses sufficient to depress the blood glucose level to normal within 6 to 12 hours. Animals were sacrificed 10 minutes, 1, 6, 24 and 48 hours after the beginning of insulin administration and livers and diaphragms were removed. The livers were sliced and incubated with C^{14}-labelled glucose, fructose, glycerol, pyruvate and acetate. Substrate utilization, total glucose phosphorylation, substrate incorporation into glycogen, into glucose and into CO_2 were measured. The marked differences between normal and diabetic liver metabolism were again demonstrated. In addition, it was found that whereas in diaphragm insulin exerts maximal effects within 10 minutes to 1 hour after its administration, in hepatic tissue insulin effects on all measurements did not begin until 6 hours, normal values being reached between 24 and 48 hours only.

This observation accounts for the inability to demonstrate effects of insulin added to liver *in vitro*, and further emphasizes the probable complex nature of insulin action. Possible implications as to treatment of diabetic acidosis will be discussed.

Protein-Binding of Thyroxine in Normal and Nephrotic Serum. J. ROBBINS, J. E. RALL and M. L. PETERMANN, New York, N. Y. (Introduced by R. W. Rawson).

The thyroxine in normal human serum is bound largely to a specific protein with electrophoretic mobility intermediate between alpha-1 and alpha-2 globulin (barbital buffer, pH 8.6). The thyroxine-binding capacity of this specific protein was studied in five normal serums. Synthetic 1-thyroxine-I^{131} was added *in vitro* in concentrations from 0.01 to 10 micrograms per ml.; and the distribution

of radioactivity among the proteins separated by paper electrophoresis was determined. The pattern of distribution changed very little until the total serum thyroxine content reached approximately two to three times normal. At higher concentrations, thyroxine was found in increasing amounts in all protein fractions, but albumin became the major carrier. Essentially all of the thyroxine was bound to protein at even the highest concentrations, as shown by ultracentrifugation. Exchange between endogenous and exogenous thyroxine was demonstrated by the addition of unlabeled thyroxine to serum containing endogenously labeled thyroxine.

The derangement in iodine metabolism in the nephrotic syndrome includes a low serum organic iodine level, and a low BMR which is unresponsive to thyroxine administration. The serum thyroxine-binding protein in three patients with nephrosis was studied as described above. At low concentrations, thyroxine was bound largely to protein resembling the normal thyroxine carrier in electrophoretic mobility and in ultracentrifugal sedimentation. Excessive amounts of thyroxine, however, were carried to a large extent in the beta globulin, and the amount associated with albumin varied directly with the albumin concentration. The thyroxine-binding capacity of the specific protein, while possibly somewhat below normal, was adequate to bind at least the normal serum thyroxine content. A reduction in this protein does not, therefore, appear responsible for the lowered serum iodine in nephrosis.

Effects of Potassium on the Renal Tubular Reabsorption of Bicarbonate. KATHLEEN E. ROBERTS, HENRY T. RANDALL and HAROLD SANDERS, New York, N. Y. (Introduced by Thomas P. Almy).

The renal reabsorption of bicarbonate and the excretion of titratable acid, potassium, phosphate, and ammonium have been studied in human subjects during metabolic (hypokalemic) alkalosis, and in dogs a) during respiratory acidosis and b) following acute reductions in glomerular filtration rate. Each patient was studied a) during a five-day control period, b) during hypokalemic alkalosis, and c) following administration of potassium. Calculations of the amount of base-bound bicarbonate reabsorbed per 100 cc./glomerular filtration rate shows that the hypokalemic patients reabsorbed 3.2 to 3.4 mEq./100 cc./glomerular filtration rate, as compared to the normal reabsorption of 2.7 to 2.9 mEq./100 cc. of GFR. Following the administration of potassium, the bicarbonate reabsorption returns to normal coincident with correction of the plasma alkalosis. During the hypokalemic period, the urinary excretion of titratable acid, ammonium, and potassium decreased in comparison to the control period. Quantitatively, the increase in absorption of bicarbonate bound base could not be accounted for by the renal tubular exchange of hydrogen, ammonium, or potassium ions for sodium.

Experiments were carried out on dogs to determine the effects of potassium on the increased bicarbonate reabsorption: a) as described in conjunction with respiratory acidosis and which has been attributed to elevation of plasma pCO_2, and b) as reported following acute reductions in glomerular filtration rate. In both situations the administration of potassium decreased the renal tubular reabsorption of bicarbonate to normal values. These studies and data to be discussed suggest that alterations in intracellular pH consequent to changes in intracellular concentrations of potassium, bicarbonate and pCO_2 condition the renal reabsorption of bicarbonate in situations of hypokalemic alkalosis and respiratory acidosis.

Affinity of Gentisate for Connective Tissues Demonstrated by Fluorescence in the Ultraviolet. BETTY S. ROOF and JOSEPH C. TURNER,* New York, N. Y.

A part of the problem of the mode of action of antirheumatic drugs concerns whether they may be actually bound by connective tissues or exert their action less directly. Fluorescence microscopy has been applied by others to the study of the distribution in vivo of a number of aromatic compounds, and the claim has been made that salicylate "lacks connective tissue affinity." However, a systematic study of the distribution of antirheumatic substances appears to be wanting.

It was decided to examine the behavior of gentisate, because 1) gentisate has a far greater intensity of fluorescence than other antirheumatic agents, 2) it has relatively low toxicity, and 3) salicylate is oxidized in part to gentisate in the body and this conversion may indeed be essential to its action.

Mice and rats were injected intravenously with solutions of gentisate and sacrificed a few minutes later. When viewed with the eye in ultraviolet light, intense fluorescence was shown by the connective tissues such as tendon, cartilage, and subcutis. Viscera and muscle fluoresced dimly or not at all.

The technique can be refined by the application of ultraviolet microscopy. Meanwhile, the finding indicates that at least one active antirheumatic drug has an affinity in vivo for connective tissue. It would appear that an extension of the studies might provide useful information about certain aspects of the metabolism of these compounds.

For example, it has been possible to show in vitro that fragments of cartilage or tendon immersed in a watery solution of gentisate for 30 minutes exhibit fluorescence that persists after washing under the tap or after standing overnight in physiological saline. This indicates that the fluorescence observed in vivo is due to binding of gentisate by some element of connective tissue.

The Arresting Effect of Heparin Upon the Development and Course of Experimental Nephrosis. RAY H. ROSENMAN, SANFORD O. BYERS and MEYER FRIEDMAN,* San Francisco, Calif.

The injection of rabbit anti-rat kidney serum into the rat induced a nephrotic state, characterized, as in man, by albuminuria, edema, ascites, lipemia, and hypercholesteremia.

Administration of heparin was found to prevent or profoundly modify the development of this nephrotic syndrome if it were given either before or concomitantly with the injection of the anti-kidney serum. Thus, complete prevention of the syndrome was accomplished in a series of rats given a *continuous* intravenous injection of heparin immediately following the injection of potent anti-kidney serum and almost complete prevention, in three different series of rats given heparin intermittently following the administration of the specific serum. Thus the marked edema, ascites, lipemia and hypercholesteremia noted in the control injected animals were not observed in the rats also treated continuously with heparin. Serum injected rats given *intermittent* injections of heparin likewise failed to exhibit edema and ascites and showed only a minimal rise in serum lipids and cholesterol as compared to the control animals. The specific suppressive effect of heparin was demonstrated by the emergence of the full-fledged nephrotic state in the experimental rats 48 hours after discontinuance of the heparin therapy.

Administration of heparin to animals already exhibiting the nephrotic state also was found to depress markedly, their lipemia and hypercholesteremia. Possible therapeutic applications to clinical nephrosis will be discussed.

Plasma Thromboplastin Antecedent (PTA) Deficiency in Man: Clinical, Coagulation, Hereditary and Therapeutic Aspects. ROBERT L. ROSENTHAL, New York, N. Y. (Introduced by Arthur M. Fishberg).

In previous studies, an hereditary, hemophilia-like disease was discovered in 2 sisters and their maternal uncle who showed prolongations in the clotting time in the range of 20 to 30 minutes and abnormal prothrombin utilization. The clotting factor deficient in these cases, designated as plasma thromboplastin antecedent (PTA), was found to differ in properties from antihemophilic globulin (AHG) and plasma thromboplastin component (PTC). PTA is essential for the normal generation of thromboplastin during coagulation.

Studies on other members of the original family of PTA deficient patients revealed that PTA deficiency can also occur to a lesser degree characterized by a normal clotting time, normal to moderately prolonged heparin clotting time and impaired prothrombin utilization. These studies also established that PTA deficiency is transmitted by or to either male or female as a simple autosomal dominant trait.

Eighteen patients in 12 families (10 females, 8 males) have so far been diagnosed as PTA deficient. Except for the original 3 cases with prolonged clotting times, the clotting times in these patients have been normal. Many of these patients have been previously diagnosed as having either thrombasthenia or vascular defects. Hemorrhagic manifestations in these cases usually follow surgery or trauma and seldom occur spontaneously. Severe hemorrhage often follows tooth extractions and tonsillectomy. Purpura and joint involvement rarely occur. The administration of stored, refrigerated plasma has been found to correct the clotting defect and is effective in controlling hemorrhage or as a prophylactic measure prior to surgery.

These studies are significant in that many instances of unexplained, abnormal bleeding can now be attributed to PTA deficiency and can be effectively treated or prevented.

Auscultatory Determination of Arterial Pressure at Wrist and Ankle. DAVID A. RYTAND* and SAMUEL H. BOYER, IV, San Francisco, Calif.

Because the usual clinical methods yield erroneously high values for arterial pressure when the standard cuff is applied to large extremities such as thighs, use of a wider cuff has been recommended. But wider cuffs are not always available, nor has optimal width been established. We have found that the standard cuff is satisfactory on the legs, as well as on the arms of even grossly obese subjects, when it is applied just proximal to ankle or wrist. Auscultation at the appropriate artery is facilitated by removal of the plastic portion of the stethoscope bell. The method is practicable in all but hypotensive patients with faint Korotkow sounds.

In 53 recumbent normal adults, average systolic pressure by auscultation was 6 mm. Hg lower in the radial than in the brachial artery, mean diastolic pressure (muffling) 6 mm. Hg higher; the discrepancy was less than 20 mm. Hg in 90% of observations (not quite simultaneous). In the posterior tibial and dorsalis pedis arteries, systolic pressure was 2 mm. Hg and diastolic pressure 5 mm. Hg above brachial levels; these values resembled those at the popliteal artery distal to a wider cuff at the thigh.

In 20 hypertensive patients, mean pressure relationships were as above, with greater individual variations. With aortic insufficiency, findings were similar but for Hill's elevation (30 mm. Hg) of posterior tibial systolic pressure. Discrepancies between muffling and disappearance of sound at diastolic levels were 3 times greater at the brachial than at the radial artery. In 3 patients with mammoth obesity, radial and ankle pressures were far below brachial ones (60–140 mm. Hg systolic, 20–85 mm. Hg diastolic); this was not so with obesity of ordinary degree. Results following operation for coarctation of the aorta were readily followed.

Clinical, Metabolic and Genetic Studies in Myotonia Dystrophica. AVERY A. SANDBERG, FAYETTE E. STEPHENS and FRANK H. TYLER,* Salt Lake City, Utah.

In personally examining the members of 15 kindreds in which myotonia dystrophica was found more than 100 affected individuals were observed. Nearly all were residents of the local rather small population, indicating that this disease is among the most common of neuromuscular disorders in this locality. Although the disease showed a wide spectrum of expression, in most cases the involvement was rather typical. The following features were usually present: myotonia, muscular atrophy in a characteristic distribution, subcapsular cataracts, gonadal in-

sufficiency, frontal alopecia, and peripheral trophic and vasomotor changes, sometimes with Raynaud's phenomenon. A high proportion of the patients had cardiac dystrophy. Progress of a barium meal through the intestine was frequently slow and irregular. Absorption of certain substances (glucose, iron) was delayed. These changes are probably the result of involvement of smooth muscle. The incidence of mental deficiency was greater than in patients with other muscular dystrophies or in the general population.

In 17 of these individuals detailed metabolic and endocrine studies were performed. Because certain of the clinical features resemble myxedema and basal metabolic rates are low, others have suggested that hypothyroidism is an etiological factor in the disorder. We have demonstrated normal I^{131} uptake by the thyroid gland in these patients; the low metabolic rate is probably due to the loss of muscle mass. Although in contrast to other muscular dystrophies, creatinuria was irregular in these cases of myotonia dystrophica, serum creatine levels were usually elevated. Adrenocortical function was normal, although the 17-ketosteroid excretion in the urine was low in patients with testicular atrophy.

Inheritance of the trait was consistent with that of an autosomal dominant. Although penetrance appeared to be complete there was an extremely wide range of expression of the trait.

Antibody Synthesis in Vitro. JAY P. SANFORD, CUTTING B. FAVOUR and ERICKA LINDSAY, Boston, Mass. (Introduced by John G. Gibson, II).

The demonstration of a humoral immune factor by Pfeiffer and Marx in 1898 and the implication of the spleen playing a major role in its production stimulated numerous investigations concerning the sites of antibody synthesis. The present studies were undertaken to obtain further data on the role of the circulating lymphocyte in relation to that of splenic cells in the immune response. The antigen used was the enzyme urease. Antibody (anti-urease) was measured by the inhibition of ammonia and of carbon dioxide release from a urea substrate by a known quantity of urease. The injection of this material into rabbits produced high levels of circulating antibody.

The determinant group in the urease to which the antibody appears directed is not that portion of the molecule which is responsible for its enzymatic action, as demonstrated by the poor "anti-urease" activity where the specific antigen-antibody complex was not removed from solution by centrifugation.

Our data clarify further the role of the circulating lymphocyte and its relationship to splenic cells in the immune response. Both the spleen and circulating lymphocytes from an immune rabbit contain a quantity of specific antibody which is released upon incubation *in vitro*. Cellular respiration may be inhibited by toluol without affecting this release of antibody. An additional quantity of antibody is produced by splenic tissue *in vitro* if metabolic activity is allowed to continue. In contrast the lymphocyte, while metabolically active, is unable to increase the amount of antibody released *in vitro*.

This circulating antibody is produced in association with plasmacytic maturation, at least within the spleen. The circulating lymphocyte may carry the antibody and be associated with its release; however, it does not have the capacity for antibody synthesis *in vitro*.

Solute Load and the Renal Osmotic Parameters of Chronically Dehydrated Men. F. SARGENT, II and R. E. JOHNSON,* Urbana, Ill.

Urine flow and urine-serum osmolar ratio (U/S) are quantitatively related so that the minimum flow at a given solute load is the asymptote for maximal U/S ratios. This parameter has been used repeatedly in discussions of water requirements, to the neglect of another measurement that is important in renal function: the urine flow at which U/S becomes 1.0. The first parameter defines the condition for maximal renal osmotic work; the second, that for minimal work. Male students, apparently free from disease, were subjected during two week periods in a comprehensive metabolic study of five months' duration to all possible combinations of the following average daily allowances: water, unlimited or 0.9 liters; sodium chloride, 0, 5 or 20 grams; nitrogen, 0, 6, 12, 17 or 24 grams; and calories, 0, 1000, 2000 or 3000. Chronic dehydration combined with high solute load resulted in daily urine flows of about 0.8 liters as a minimum, and U/S osmolar ratios as high as 5.9; in contrast, when solute load was low (pure carbohydrate or high fat diet) minimum daily urine flow was about 0.25 liters and maximum U/S about 4.2. With unlimited water and high solute load, the U/S ratio approached 1.0 as an asymptote at daily flows of 2.5 liters or over; in contrast, with unlimited water and a low solute load U/S reached 1.0 with daily urine flows of 0.8 liters and then approached an asymptote of less than 0.1 with greater flows. We conclude that the urine flow for minimal renal osmotic work increases with increasing solute load and is related importantly to water requirements and renal function during different dietary regimens.

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Motion Picture Studies of Ventricular Muscle Dynamics in Experimental Localized Ischemia, Correlated with Myocardial Oxygen Tension and Electrocardiograms. JOHN J. SAYEN, WARNER F. SHELDON, GEORGE PEIRCE and PETER T. KUO, Philadelphia, Pa. (Introduced by Charles C. Wolferth).

It is well known that systolic bulging of muscle occurs in the distribution of an acutely occluded coronary artery. Disturbed muscle contraction is an essential index of severe myocardial ischemia. Acute localized ischemia was studied in dogs by continuous recording of polarographic oxygen tension and simultaneous intramyocardial electrocardiograms from ten platinum electrodes inserted in the left ventricle to various depths. The relative motions of these electrodes could then be analyzed from motion picture records by measuring the distances between

markers attached to their glass shafts at the epicardial surface. Events were synchronized by a flashing neon bulb, triggered by the first upward deflection of each QRS complex in an intramyocardial electrocardiogram. Color film permitted rough estimations of epicardial arteriovenous differences and muscle discoloration.

Results: Non-ischemic hearts yielded various types of muscle contraction record. The areas of earliest shortening reduced presystolic muscle segment lengths by as much as ⅓ within 0.25 seconds of electrical systole. Other areas showed delayed shortening or very slight changes. Regardless of its control contraction pattern, muscle in severely ischemic areas was stretched rapidly just after electrical systole, reaching a maximum length increase about 0.25 second thereafter. Less severely ischemic muscle failed to shorten or stretched after partial systole. Muscle beyond the ischemic area was unaffected or somewhat increased its amplitude of contraction.

Coronary occlusion produced rapid falls of oxygen tension beginning within six to eight heart beats. Muscle contraction disturbance could be detected as early as the eighth heart beat. An extrasystole regularly occurred within fifteen heart beats and about this time the intramyocardial electrocardiogram began to show abnormality. Epicardial electrocardiograms became abnormal later, often not until oxygen tension had fallen profoundly. Very severe acute coronary narrowing resembled occlusion in its consequences. Slighter narrowing could cause reduced oxygen tension and intramyocardial electrocardiographic abnormality without measurably changing epicardial electrocardiograms or muscle contraction.

The Concentration of Copper and Ceruloplasmin in Maternal and Infant Plasma at Delivery. I. HERBERT SCHEINBERG,* CHARLES D. COOK and JANET A. MURPHY, New York, N. Y.

At delivery, maternal plasma has a concentration of copper about 5 to 6 times that of infant plasma. Almost all of the copper in the plasma of normal adult human beings is irreversibly bound within the protein molecule, ceruloplasmin, whose molecular weight is 150,000. It seemed unlikely that equilibrium with respect to diffusion across the placental membrane would be approached by a molecule of this size, whereas non-ceruloplasmin copper might be expected to be in such equilibrium. This study was undertaken to investigate these suppositions.

The concentrations of total copper, of ceruloplasmin and of non-ceruloplasmin copper were measured in maternal plasma, taken immediately before delivery, and in plasma from the umbilical cord. In twelve pairs of mothers and infants, the average level of total copper in maternal plasma was 216 (range: 118–302), and that in cord plasma was 36 (range: 12–67) gamma/100 ml. In the same subjects, the average level of ceruloplasmin in maternal plasma was 55.6 (range: 39.4–89.0), and that in cord plasma was 6.5 (range: 1.8–13.1) mg./100 ml. Since 0.34% of ceruloplasmin is copper, the maternal plasma contained an average of 189 and the cord plasma contained an average of 22 gamma/100 ml. of ceruloplasmin copper. In fifteen pairs of mothers and infants the average levels of non-ceruloplasmin copper were 10.7 (range: 5–15) and 8.5 (range: 0–16) gamma/100 ml. for maternal and infant plasma, respectively. Non-ceruloplasmin copper appears to approach equilibrium with respect to diffusion across the placenta since the average difference between maternal and infant values is not statistically significant by the usual criteria. In contrast, the fact that ceruloplasmin copper is not in such equilibrium accounts for the large gradient of copper between maternal and infant plasma.

Neurogenic Factors in Coronary Artery Syndromes. I. Experimental Production of Electrocardiographic Patterns Simulating Acute Myocardial Infarction, Coronary Insufficiency and Cardiac Arrhythmias by Stimulation of Autonomic Nerves. SIDNEY SCHERLIS, R. ADAMS COWLEY and GEORGE SCHIMERT, Baltimore, Md. (Introduced by Theodore E. Woodward).

Psychogenic factors are known to precipitate the anginal syndrome of coronary insufficiency although the nervous pathways have not been clearly defined. Furthermore, thrombosis of the coronary arteries and ultimate myocardial infarction are said to result from arteriosclerotic changes, yet the factors culminating in the acute episode have not been clearly elucidated. Based upon well founded clinical considerations, we accepted the premise that neurogenic factors may precipitate episodes of coronary insufficiency and even occlusion in patients with predisposing atherosclerosis. The dog has been employed as an experimental model to investigate these factors.

After transthoracic thoracotomy, the left vagus and sympathetic nerves were exposed and stimulated at various sites by faradic current. Uniform electrocardiographic changes simulating patterns typical of acute myocardial infarction occurred promptly when isolated vagal branches to the cardiac plexus were stimulated, viz.: elevation and coving of ST segments and T wave inversion in certain conventional leads. Less frequently, ST segments were depressed and T waves became sharper with increased amplitude simulating the pattern of acute coronary insufficiency. Stellate ganglion stimulation likewise produced the latter type of electrocardiographic response, and minimized the electrocardiographic effects of preceding "vagal" stimulation. Stimulation of vagal cardiac nerve pathways resulted in cardiac arrhythmias: auricular flutter-fibrillation, A-V nodal rhythm, extrasystoles, ventricular standstill, tachycardia and fibrillation. On one occasion, following brief stimulation, an "acute coronary-like" pattern was noted, followed immediately by ventricular fibrillation and death.

These striking electrocardiographic changes hitherto unreported following autonomic nerve stimulation suggest either 1) interference with coronary blood flow or 2) alteration of myocardial function by metabolic mechanisms.

These preliminary findings are discussed relative to theories of pathogenesis of coronary occlusion. Blocking

action by pharmacological methods has received consideration.

A new surgical approved therapy of coronary disease based upon these factors is under investigation.

Erythropoietic (Congenital) Porphyria: A Blood Dyscrasia Characterized by a Peculiar Disturbance of the Normoblasts. RUDI SCHMID, SAMUEL SCHWARTZ and R. DOROTHY SUNDBERG, Minneapolis, Minn. (Introduced by C. J. Watson).

Recent studies have shown that the photosensitive forms of porphyria include two fundamentally different disturbances of porphyrin metabolism, one related to hemoglobin formation during erythropoiesis (Porphyria erythropoietica), the other a liver dysfunction (Porphyria hepatico). Erythropoietic (congenital photosensitive) porphyria is a rare inherited disease manifest at birth or during early life. Critical review of the reported cases in the literature reveals only 34 instances, in which the diagnosis could be established beyond reasonable doubt. They were 19 female and 15 male children, the great majority having erythrodontia, hypertrichosis, hemolytic anemia and splenomegaly.

Bone marrow and blood preparations of five of these patients, were available for study. In all instances, excessive amounts of porphyrins were present in marrow and circulating erythrocytes. Using fluorescence microscopy, most of the porphyrin was found in the *nuclei* of the normoblasts in the marrow. By studying and photographing the same group of marrow cells, first in the fluorescence microscope, then with absorption spectroscopy and, finally, by using a specialized staining technique, the existence of a *normal* and an *abnormal* line of normoblasts was discovered. Cells of the abnormal variety exhibit high concentrations of porphyrins in their nuclei as well as characteristic intranuclear inclusions, containing hemoglobin. This, in all probability, represents the inborn metabolic error of this disease. The normal cell type shows neither porphyrin fluorescence nor nuclear inclusion bodies.

The ratio of normal to abnormal normoblasts in the bone marrow varies in different patients from 1:3 to 3:1, but it does not appear to be related to the severity of the clinical manifestations. Splenectomy performed in 3 cases did not alter this ratio, but it did correct the hemolytic anemia. As a result, the normoblastic hyperplasia and hence porphyrin formation and excretion decreased.

The Relation of Life Situations to Metabolic Balance. W. W. SCHOTTSTAEDT, Oklahoma City, Okla. (Introduced by Stewart Wolf).

Three adult males without known renal or cardiovascular disease have been studied under rigid control on a metabolic ward for periods up to eight weeks in duration. Records were kept of events occurring on the ward and of the attitudes and emotions or feeling states aroused in the patients by these events. No stress interviews or contrived situations were introduced. The subjects were maintained on uniform intake of water, electrolytes and diet throughout the period of study and daily determinations of urinary nitrogen, electrolytes, and water excretion were made.

Marked variations in rates of excretion were observed despite the rigid regimen. In a subject on whom urine specimens were obtained four times a day, the evening urine volumes varied from 0.49 to 2.88 cc/min; sodium excretion from 34 to 278 μEq./min; potassium from 52 to 182 μEq./min; nitrogen from 3.23 to 13.83 mg/min; and corticoids from 0.35 to 1.53 μEq./min despite constant intake and constant exercise. 24 hour urine volumes varied from 630 to 1680 cc and urinary nitrogen varied from 7.02 to 13.00 gms. All marked variations from the average rates of excretion were easily correlated with troublesome events occurring spontaneously. For example, anxiety was induced by the illness of other patients and anger because a meal was served cold instead of warm. Tension was induced by brooding over past health and depression because of the failure of expected visitors to appear.

The study has shown that careful control of intake and activity is not adequate to assure a steady metabolic state. Marked variations in urinary excretion of nitrogen, electrolytes and water correlated with events occurring during ward routine and with the emotions and feeling states aroused by these events.

The Osmotic Activity of Cells of Mammalian Tissues. IRVING L. SCHWARTZ and EUGENE L. OPIE, New York, N. Y. (Introduced by Vincent P. Dole).

Slices of liver and kidney cortex, taken from rats immediately after death and immersed in a series of salt solutions, swell if the concentration is less than about twice that of extracellular fluid, and shrink if the concentration is greater. From these results it is inferred that the osmotic activity within the cells of the slice is about twice that of the solutes in normal extracellular fluid.

This conclusion, however, depends on the assumption that salt remains extracellular during the period of measurement. Evidence to support this assumption has been obtained in the following experiments: fresh slices of liver, kidney cortex, and corium were weighed, immersed for periods of time from 1 to 60 minutes in solutions of 0.1 to 1.0 N NaCl, reweighed to measure the gain or loss of water, and then analyzed for electrolytes. It was found that the entry of sodium into the tissues was independent of the movement of water, and simply proportional to the concentration of sodium in the external medium. The results indicated that the medium quickly came into equilibrium with $20 \pm 2\%$ of the liver mass, $31 \pm 2\%$ of the kidney, and $49 \pm 1\%$ of the corium—values which are close enough to those accepted for extracellular space to suggest that the effect of immersion within the first ten to fifteen minutes is to equilibrate the bath and the interstitial fluid. Later, the intracellular concentration of sodium rises, but at the end of the first ten minutes only the interstitial fluid has equilibrated. Therefore, the isosmotic solution can be taken as a measure of the osmotic activity of cells in the tissue slices.

Effect of Cortisone on Intracellular Peptidase Activity. THEODORE B. SCHWARTZ, Durham, N. C. (Introduced by James V. Warren).

Dipeptidase activity of surviving diaphragm has been used as a model to represent intracellular protein catabolic processes. This enzymic activity is determined by measuring the rate at which glycylglycine is hydrolyzed by viable isolated rat diaphragm tissue. The enzyme is strongly activated by cobalt and inhibited by cysteine, and these variations have been demonstrated both *in vitro* and *in vivo*. A 50% increase in dipeptidase activity of surviving diaphragm followed the administration of cortisone, 5 mgm. a day, over a 5-day period. This hormonal effect requires the presence of intact cells since it was not demonstrable in rat muscle extracts or homogenates. The mechanism by which such *biologic* activation occurs is being explored; and there are indications that this cortisone-induced enhancement of peptidase activity of isolated diaphragm is *not* mediated by way of increased availability of cobalt ion since, when the experiment was repeated with optimal amounts of cobalt added *in vitro*, dipeptidase activity of cortisone-treated animals remained greater than normal.

These observations are consistent with the view that the protein catabolic effect of adrenal steroids is, in part, the result of enhanced intracellular, proteolytic activity. This hormonal effect is demonstrable only in the presence of viable, intact cells and apparently does not operate through metal activation.

The Dependence of Renal Sodium Reabsorption on Hydrogen Exchange. WILLIAM B. SCHWARTZ* and ARNOLD S. RELMAN, Boston, Mass.

Net tubular transport of water and electrolytes has been measured in well-hydrated anesthetized dogs given large doses (500 mgm./kgm.) of acetazoleamide, a potent carbonic anhydrase inhibitor. Frequently, half or more, and on several occasions two thirds, of the filtered sodium was excreted together with eighty to ninety per cent of filtered bicarbonate, twenty to forty per cent of filtered chloride and an amount of potassium at least equal to, and up to, eighty-six per cent greater than the filtered load of potassium.

During this brief profuse diuresis, urine volume was one-half to two-thirds of the volume of the glomerular filtrate and U/P osmolar ratios approximated unity. Filtration rate was usually unaffected or slightly reduced.

Maximal depression of bicarbonate reabsorption (to rates of .1 to .6 mEq./min./100 cc. GFR) was observed with large doses of the inhibitor at widely varying levels of plasma bicarbonate (19–75 mEq./L.) and blood pCO_2 (25–100 mm. Hg). With lower doses of inhibitor (10–50 mgm./kgm.), one-third to one-half the normal load of bicarbonate can be diverted into the urine with little or no consistent effect on chloride excretion; the latter was consistently increased only with maximal dosage.

These observations are consistent with the hypothesis that all bicarbonate reabsorption is accomplished by tubular exchange of hydrogen for sodium and that carbonic anhydrase is required to catalyze this reaction if the rate of reabsorption is to be more than minimal. The data further suggest that most, if not all, sodium reabsorption is effected by hydrogen-sodium exchange. The large increment in chloride excretion seen only with maximal doses of inhibitor is probably secondary to the diuresis of sodium which results when the rate of tubular hydrogen ion generation falls significantly below the rate of filtration of sodium chloride.

Potassium Intoxication During and Immediately Following Respiratory Acidosis. BELDING H. SCRIBNER, GEORGE M. BOGARDUS, KENNETH FREMONT-SMITH and JAMES M. BURNELL, Seattle, Wash. (Introduced by Robert S. Evans).

Unexplained cardiac arrest and death have been observed during and immediately following the respiratory acidosis of general anesthesia. These studies indicate that potassium intoxication associated with respiratory acidosis may be an important factor.

Dogs were made acidotic for 2–5 hours with a mixture of 30% CO_2 and 70% oxygen. Blood pH fell promptly and remained between 6.7 and 6.9. Balance studies revealed that during the acidosis 5 to 15 milliequivalents of potassium were transferred per hour from the cells into the extracellular space. Arterial serum potassium always rose at a rate which varied inversely with urinary excretion. Most animals survived serum potassium levels as high as 9 to 10 mEq./L. without electrocardiographic changes as long as they were maintained in acidosis. Immediately upon return to room air, blood pH rose abruptly and electrocardiographic evidence of potassium intoxication appeared often progressing to cardiac arrest and death. Prompt reinstitution of respiratory acidosis was frequently lifesaving.

The appearance of potassium intoxication with the relief of respiratory acidosis was related to at least 2 factors: 1) There was a transient further increase in the serum potassium level of 0.5 to 1.0 mEq./L. when the acidosis was terminated. 2) Potassium loading studies revealed that potassium intoxication in non-acidotic dogs occurred at serum concentrations 2 to 4 mEq./L. lower than in dogs in respiratory acidosis.

These data suggest that certain unexplained anesthetic deaths may be due to potassium intoxication because: 1) Respiratory acidosis causes hyperkalemia. 2) Relief of acidosis causes further transient hyperkalemia. 3) There is less tolerance to hyperkalemia at normal blood pH than during respiratory acidosis.

The Relation of Hypokaliemic Alkalosis Induced by Adrenal Steroids to Renal Acid Secretion. DONALD W. SELDIN,* FLOYD C. RECTOR, JR., NORMAN CARTER and JOHN COPENHAVER, Dallas, Tex.

The cause of the alkalosis which develops in animals rendered potassium deficient by administration of DOCA and sodium has not been clearly elucidated. To clarify the role of the kidneys in the alkalotic process, rats were tube-fed standard electrolyte-deficient diets for 14 days

and treated with various combinations of electrolytes and DOCA. Urine was analyzed for pH, titratable acid, and ammonium. Muscle and serum electrolytes were measured. Activities of glutaminase, glycine oxidase, leucine oxidase, and carbonic anhydrase were determined in the kidneys.

Rats receiving sodium, DOCA, but no potassium, developed severe systemic alkalosis and excreted approximately 8 meq. more acid, primarily as NH_4, than corresponding control animals. The alkalosis must therefore be, in part at least, a consequence of the renal loss of acid. Associated with this accelerated ammonium excretion renal glutaminase activity increased from 788 ± 39 μM NH_3/100 mgm. dry kidney/hr. in control animals to 1842 ± 445 in animals rendered alkalotic with sodium and DOCA. Neither renal carbonic anhydrase nor ammonia-producing enzymes other than glutaminase changed significantly with increased acid secretion.

The increased glutaminase activity was not a consequence of any direct action of DOCA or sodium on the renal tubules, since rats given either DOCA or sodium salts, but with sufficient potassium to prevent potassium deficiency, developed neither alkalosis nor increased glutaminase activity.

These findings suggest that hypokaliemic alkalosis produced by DOCA and sodium is, in part at least, a consequence of the renal loss of acid, principally as ammonium. Accelerated ammonium excretion, in turn, apparently results from the activation of renal glutaminase. This enzymatic adaptation was not a result of the direct effect on the tubule of sodium or DOCA, but occurred only if potassium deficiency supervened. It is suggested that the adaptation of glutaminase may be stimulated either by potassium depletion or intracellular acidosis.

Triiodothyronine Administered Orally in the Treatment of Myxedema. HERBERT A. SELENKOW and SAMUEL P. ASPER, JR.,* Baltimore, Md.

Recent investigations indicate that triiodothyronine is the most potent form of the thyroid hormone known. The *levo* isomer of triiodothyronine, administered parenterally to patients with myxedema, possesses three to four times the calorigenic effect of *l*-thyroxine and exhibits all the metabolic properties ascribed to the thyroid hormone.

In the present study the calorigenic activities of *l*-triiodothyronine, *dl*-triiodothyronine and sodium *l*-thyroxine administered orally were determined in six patients with myxedema. The daily dose required to maintain euthyroidism was 70 to 105 micrograms of *l*-triiodothyronine, 200 to 300 micrograms of *dl*-triiodothyronine and 300 micrograms of sodium *l*-thyroxine. All patients receiving triiodothyronine became clinically euthyroid within ten to fourteen days. During this period each patient exhibited a six to ten pound loss in weight, a decrease in the serum cholesterol to normal levels and an increase in basal metabolic rate to normal values. In addition, during administration of either *l*- or *dl*-triiodothyronine, levels of serum protein-bound iodine were in the range of myxedema at times when the patients were completely euthyroid. This has also been noted following parenteral administration of triiodothyronine. It is apparent that the level of the serum protein-bound iodine is not indicative of the metabolic status of athyreotic patients receiving triiodothyronine as judged by present standards of the normal range for this determination.

Triiodothyronine administered orally was uniformly effective in alleviating myxedema. The *dl*-isomer of triiodothyronine had approximately one-half the calorigenic effect of an equivalent dose of *l*-triiodothyronine; this suggests that the *d*-isomer probably is calorigenically inactive. The *l*-isomer of triiodothyronine possessed three to four times the calorigenic effect of sodium *l*-thyroxine. Triiodothyronine has the therapeutic property of acting rapidly in minute doses. Like thyroxine, it is a chemically pure synthetic form of the thyroid hormone, obviating standardization.

The Participation of Fibrin in the Activation of the Fibrinolytic Enzyme of Plasma. SOL SHERRY,* Cincinnati, O.

A number of observations have suggested that, in native plasma, under conditions conducive to the activation of plasminogen (the precursor of the fibrinolytic enzyme), fibrinolysis proceeds at a much faster rate than fibrinogen breakdown. However, plasmin, the active enzyme, in purified systems attacks fibrinogen and fibrin at the same rate and its activity on these substrates appears to be directly related to its general proteolytic properties. To explain this paradox, Ratnoff has suggested an accelerated activation of plasminogen in the presence of a fibrin clot.

We have obtained direct evidence, in vitro, that the streptokinase activation of plasminogen in plasma is markedly accelerated by the presence of a fibrin clot. The observations indicate that activation rapidly occurs on the fibrin where considerable amounts of plasminogen and streptokinase are adsorbed. The local activation of the enzyme on the fibrin results in rapid fibrinolysis, followed by release of the activated enzyme into the surrounding plasma, where it is slowly inhibited. In vivo observations, following the intravascular injection of streptokinase into dogs with intraarterial thrombi resulted in a very small increase in circulating proteolytic activity and minor changes in the plasminogen and fibrinogen concentrations of the plasma, yet produced rapid liquefaction of normally clotting blood specimens, and significant dissolution of the experimental thrombi. In sharp contrast, when plasminogen was completely activated *in the plasma*, in vivo, by another mechanism, large amounts of circulating proteolytic activity appeared, the plasminogen disappeared, and an incoagulable state rapidly resulted. Dissolution of the experimental thrombi, however, was similar to the streptokinase treated animals.

The observation that fibrin provides a favorable surface for the mediation of the activation of plasminogen by streptokinase suggests that this phenomenon may be in-

volved in naturally occurring fibrinolysis, and may also explain the special fibrinolytic activity observed in patients following streptokinase injections.

The Mechanism of Pigmentation in Addison's Disease. KAZUO SHIZUME, AARON BUNSEN LERNER and THOMAS B. FITZPATRICK, Portland, Ore. (Introduced by Arthur W. Frisch).

Clinical and experimental evidence will be presented to show that the hyperpigmentation of Addison's disease is probably due to an increase in the release of the melanocyte stimulating hormone (MSH) from the pituitary gland as a result of a decrease in adrenalcortical function.

Administration of MSH to four persons produced an increase in pigmentation. After discontinuation of the hormone, pigmentation gradually decreased, and the skin returned to its previous color.

Blood and urine levels of MSH are increased in Addison's disease. When cortisone given for treatment is withdrawn, urinary output of MSH is increased. The hyperpigmentation of Addison's patients, as well as the normal pigmentation of frogs, decreases following administration of cortisone. Cortisone probably inhibits the production of MSH by the pituitary gland.

Commercial ACTH preparations contain fairly large amounts of MSH. The hyperpigmentation resulting from long-term treatment with ACTH may result from the action of MSH present as a contaminant in the ACTH preparation.

Demonstration of Antivessel Agents in Serum of Patients with Anaphylactoid Purpura and Periarteritis Nodosa. MARIO STEFANINI* and IRMA B. MEDNICOFF, Boston, Mass.

Two cases were observed with associated acute idiopathic thrombocytopenic purpura and Henoch-Schönlein's anaphylactoid purpura. In one, a platelet isoagglutinin could be detected. In view of the recently emphasized immunologic nature of idiopathic thrombocytopenic purpura, search was conducted for agents in the serum of these patients capable of reacting with vascular elements. Decalcified serum in both instances contained precipitin against stable, saline-soluble antigen prepared from aortic intima and from total aorta of newborn infants. Complement was fixed in the course of this reaction. With separation by means of paper electrophoresis in one case (Dr. Carlos Guzman L.) the active fraction was found to be γ-globulin. The agent was relatively labile during storage (inactivated after 10 days, even at freezing temperature), and destroyed at 60° C.

A standard preparation of the aortic antigen was made, containing 20 mgs. N%. With this antigen, decalcified sera gave positive precipitation in 6 out of 8 cases of anaphylactoid purpura, 3 out of 4 cases of periarteritis nodosa. Test was negative in acute idiopathic thrombocytopenic purpura (5 cases) and lupus erythematosus (3 cases). Antigens were prepared from intimal and from adventitial and elastic layers of aorta. Perhaps due to difficulties in separating various layers of the aorta, positive sera reacted equally with both antigens. Plasma from two patients with anaphylactoid purpura was injected into compatible normal individuals. No purpura, no positive tourniquet test developed, but the antivessel factor was transmitted to the recipient.

These results appear to indicate the role of circulating antivascular agents in the pathogenesis of vascular damage in anaphylactoid purpura and periarteritis nodosa. It is likely that these factors develop as immunologic response (antibodies), since in experimental animals one obtains antivessel serum by administration of heterologous aorta constituents.

Disappearance from Serum of I^{131}-Labeled L-Thyroxine and L-Triiodothyronine in Euthyroid Subjects. KENNETH STERLING, JOYCE LASHOF and EVELYN B. MAN, New Haven, Conn. (Introduced by Philip K. Bondy).

Radioactively labeled l-thyroxine or l-triiodothyronine in tracer amounts was injected intravenously in nine euthyroid hospital patients and two normal volunteers. Serum butanol extractable iodines were normal.

Patients received an excess of non-radioactive iodine during studies to minimize thyroid uptake and reutilization of radioiodine liberated on degradation of the administered compounds.

Serum radioactivity was measured on blood samples drawn ten minutes after injection, and daily for two weeks. There was a rapid initial fall, attributed to a distribution phase. Within two days after injection of radiothyroxine, the serum radioactivity declined more gradually, assuming a straight line on semilogarithmic plot. This slow exponential component of the disappearance curve was interpreted as indicating thyroxine turnover, or the rates of formation and degradation, which in a steady state should be identical. The mean half-time of thyroxine turnover in six subjects was 6.5 days.

The disappearance curve observed after injection of labeled triiodothyronine was much more rapid, with a mean half-time of 2.6 days in four subjects. Successive studies with l-thyroxine and l-triiodothyronine in one individual clearly demonstrated the more rapid disappearance of the latter compound.

Studies on Hydralazine: Evidence for a Peripheral Site of Action. ALBERT STUNKARD, LOTHAR WERTHEIMER, and WALTER REDISCH, New York, N. Y. (Introduced by David Seegal).

An attempt was made to determine the site of action of the antihypertensive agent, hydralazine, by measurements in man of blood flow in intact and denervated extremities. Control studies of 4 patients in whom the nervous system was intact established that intravenous administration of hydralazine caused either no change or slight decrease in skin temperature, and blood flow as measured by plethysmography of the limb. The legs of 4 patients with spinal cord transection, however, showed significant increases in both skin temperature and blood flow, while the upper extremities with intact innervation responded to hydralazine administration in the usual manner.

When denervation of the limb was achieved by unilateral sympathectomy, hydralazine again increased blood flow to the denervated extremity (the leg) while reducing it in the intact leg. These observations suggested that hydralazine might act at the blood vessel wall itself. The possibility that the action might represent adrenergic blockade was excluded by the failure of hydralazine to decrease the hypertensive and bradycardic effects of norepinephrine infusion. Furthermore, no effect on the cold pressor response was observed following doses of hydralazine large enough to lower blood pressure. It was concluded that hydralazine could act directly upon the blood vessel wall, and that this might be a major site of action.

Catheterization Studies on the Bronchial and Pulmonary Vascular Systems in Dogs; With Special Reference to the Chemotherapy of Lung Cancer. R. D. SULLIVAN, T. C. ALFORD and D. A. KARNOFSKY,* New York, N. Y.

Polyethylene catheters were introduced at thoractomy, singly and in combination, into the right bronchial artery and into various branches of the pulmonary arteries and veins. The catheters were brought out through the chest wall and remained *in situ* for periods as long as three months. By injecting Diodrast, serial angiograms were obtained under various experimental conditions in studies on 23 dogs. This catheterization technique may be applied to respiratory physiologic studies.

Dye injected into the bronchial or pulmonary arteries demonstrated the arborizations of these vessels, and was rapidly cleared. After ligating the pulmonary artery and vein to a lobe, dye injected into the lobe artery or vein pooled in the vessels of the occluded lobe; however, 3 days later, dye given in the same manner, filled the bronchial arterial tree, indicating that collateral channels had opened between the pulmonary and bronchial vessels. Additional collateral channels appeared between the bronchial artery and the systemic circulation. Thus, it is possible to distribute dye throughout the bronchial arterial system by ligating a pulmonary vein, and injecting dye into the pulmonary artery to the same lobe.

Parallel procedures were performed with nitrogen mustard HN_2, 1 mg/kg, injected into the pulmonary and bronchial vessels. No local injury resulted from the injection of HN_2 into the unaltered pulmonary artery, whereas introduction into the bronchial artery severely injured the entire tracheo-bronchial tree and hilar structures, including the lymph nodes. Similar effects were produced by injecting HN_2 into the pulmonary artery three days following ligation of the pulmonary artery and vein. In contrast instillation of HN_2 through a bronchoscope into a major bronchus produced edema, hemorrhage and focal necrosis in the pulmonary parenchyma only. Since lung cancers and their regional metastases derive their blood supply from the bronchial artery, methods for modifying the pulmonary circulation at exploratory thoractomy enhance the opportunities for the local chemotherapy of these neoplasms.

A Forty-Eight Hour Procedure for the Determination of Post-Transfusion Survival of Erythrocytes from Preserved Blood, Employing Radioactive Chromium-51 (Cr^{51}). TIMOTHY R. TALBOT, JR., MARY SAX and ALICE CARY, Philadelphia, Pa. (Introduced by Thomas Fitz-Hugh, Jr.).

The method herewith presented obviates the need for blood volume determination and requires only forty-eight hours to give an accurate measure of post-transfusion survival of erythrocytes.

We have found that 25 to 50 ml. of Cr^{51} labelled erythrocytes can be injected easily in less than one minute. Therefore, observations can be made during the first few minutes after injection, followed by serial observations during the first hour. Samples are then taken at 2, 4, 6, 24, and 48 hours.

The shape of the "disappearance curve" after the injection of preserved, labelled erythrocytes is tri-phasic, due to: (a) mixing time, (b) a "rapid" phase, and (c) a "slow" phase. The curve then becomes identical in slope with that seen after the injection of fresh cells.

A "destruction gradient" can be measured between the value at the end of mixing time and the value at the end of the "slow" phase, which usually occurs at about 48 hours.

The per cent non-viable cells is equal to the "destruction gradient." This has been confirmed as follows:

(a) Total circulating hemoglobin was determined in several individuals with Cr^{51}, as described by Gray and Sterling. These same individuals were then given about 40 ml. of labelled erythrocytes from preserved blood, and the total circulating hemoglobin again calculated, using the end of mixing time as the value representing dilution. These two methods agreed within an average of 4%, with a maximum difference of 7%.

Radioactivity was measured as counts per minute per gram of hemoglobin. Thus any error inherent in using hematocrits was eliminated.

(b) 450 ml. aliquots of the same preserved blood as that used above were transfused into recipients whose total circulating hemoglobin had been previously determined as described above (Gray, S. J., and Sterling, J., J. C. I. 29, 1614, 1950.) The per cent survival was determined using Ashby counts. These values agreed within 10 % of those calculated from the "destruction gradient."

The Participation of the Erythrocyte in the Electrolyte Changes Induced by Intravenous Salt Loading. BEVERLY T. TOWERY and HOWARD E. MORGAN, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Plasma and erythrocyte concentrations and urinary excretion of sodium, potassium, chloride and water were studied in 15 normal young adults before and after a 60-minute infusion of 5 per cent sodium chloride. A constant oral water intake, established prior to infusion, was continued throughout the period of observation. A comparison is made betwen the pre-infusion period of diuresis and the second post-infusion hour. Erythrocyte

concentrations of electrolytes were determined by an indirect method.

The pre-infusion erythrocyte sodium concentration tended to vary inversely with the plasma sodium concentration, but a definite relationship has not been established. The initial plasma potassium was maintained within a narrow range despite varying red cell concentrations.

After the infusion, the changes in concentration of erythrocyte sodium and potassium bore a relationship to their initial level; high values decreased, low values rose. A correlation of the changes in erythrocyte sodium with the changes in erythrocyte potassium revealed that these ions moved in the same direction.

The change in potassium clearance was inversely related to the pre-infusion clearance of this ion and did not correlate with the change in plasma or erythrocyte concentration thereof.

The rise in chloride clearance was closely correlated with the rise in sodium clearance, but was not related to changes in plasma chloride concentration. The alteration in sodium clearance varied inversely with the change in plasma sodium concentration. This appeared to be the result of cellular and renal changes rather than the cause. A direct relationship of questionable statistical significance was found between the changes in erythrocyte sodium concentration and increments in sodium clearance. These data suggest that cellular ionic exchanges, similar to those exhibited by the erythrocyte, are important in the regulation of the renal clearance and plasma concentration of sodium.

A Comparison of the Metabolism of Rb^{86} and K^{42} Following Simultaneous Injection into Man. MALCOLM P. TYOR and JAMES S. ELDRIDGE, Oak Ridge, Tenn. (Introduced by George T. Harrell).

The physical characteristics of K^{42} (t ½ 12.4 hr) have limited its widespread use.

Studies indicate that rubidium may replace potassium in several biological systems. The physical characteristics of Rb^{86} (t ½ 19 d) are suitable for tracer studies in man.

Simultaneous intravenous injections of K^{42} and Rb^{86} were given to four patients with leukemia and carcinoma. Stable rubidium injected varied between 0.25 meq and 1.52 meq to determine any effect of "carrier"; potassium injected was 0.07 to 1.04 meq.

Multiple samples of plasma, red cells, and urine were obtained from all patients; white cells were separated in one patient. Each sample was assayed in a well scintillation counter. Counts of Rb^{86} and K^{42} were determined by taking advantage of their half lives, and analyzed statistically.

The per cent of total counts of Rb^{86} and K^{42} in each sample of plasma, red cells, and urine was relatively constant within each patient. When the ratio of Rb^{86} to K^{42} in the plasma and red cells of individual patients was compared to the ratio of the mixture injected, they were found to be similar. The ratio for white cells also agreed closely with that of the administered dose.

In contrast, the ratio of Rb^{86} to K^{42} in the urine of each patient was constantly lower than in the mixture injected. The lower values, however, bore a consistent relationship to the ratio of the dose injected in each patient. The ratios of per cent total counts Rb^{86} in urine to the per cent total counts Rb^{86} injected were similar in all patients.

Within individual patients the data indicate a constant ratio of Rb^{86} and K^{42} in plasma, red cells, white cells, and urine. The ratio is similar to that of the injected dose in all fractions except urine, which showed less Rb^{86} and more K^{42}.

Studies of Serum and Gastric Factors in the Etiology of Pernicious Anemia: A Proposed In Vitro Test for Intrinsic Factor Activity. WALTER G. UNGLAUB and O. NEAL MILLER, New Orleans, La. (Introduced by Grace A. Goldsmith).

Normal human gastric juice, collected following histamine stimulation, markedly enhances the vitamin B_{12} combining activity of serum when mixtures of serum, gastric juice, and added free vitamin are incubated together. This effect depends upon the ratio of gastric juice to serum, as well as the initial concentration of the added free vitamin, and is greatly in excess of the enhancement predicted for simple mixtures. Gastric juice from patients with pernicious anemia in relapse or in remission possesses this potentiating activity to a much smaller extent than does an equal volume of gastric juice from normal persons.

Incubation of normal gastric juice with serum from patients with pernicious anemia does not potentiate the B_{12} combining activity as much as incubation with normal serum. Gastric juice from patients with pernicious anemia enhances the combining activity of pernicious anemia serum only slightly when compared with normal gastric juice.

Studies in one patient with pernicious anemia before and after adequate oral therapy with a commercial preparation containing vitamin B_{12} and an intrinsic factor concentrate suggest an improved *in vitro* combining activity of the serum following therapy.

These observations lend support to the concept of the participation of a "serum factor" in the etiology of pernicious anemia. The techniques employed in this study have been applied to the *in vitro* evaluation of the anti-pernicious anemia activity of various intrinsic factor concentrates and they appear to give a valid test for intrinsic factor activity. Megaloblastic anemias other than pernicious anemia are being investigated with this technique.

The Relationship of Leukocyte Alkaline Phosphatase to Pituitary-Adrenal Activity. WILLIAM N. VALENTINE,* JAMES H. FOLLETTE, BARTON E. HARDIN and WILLIAM S. BECK, Los Angeles, Calif.

Unit cell leukocyte alkaline phosphatase activity frequently increases several fold in the presence of such diverse circumstances as infection, trauma, vascular emergencies, surgery and diabetic acidosis in which pituitary-adrenal hyperactivity may be a common de-

nominator. In patients studied before and after surgery leukocyte phosphatase uniformly rose stepwise to peak values averaging four times the pre-surgery level 72 hours after the procedure, remained elevated in the presence of complications, and returned to baseline over the next several days in uncomplicated procedures. A similar four fold increase occurred in each of five subjects receiving Acthar Gel for a 72 hour period with return to normal by 72 hours after stopping medication. Oral and intramuscular cortisone produced no or small responses over a similar period. Phosphatase activity required at least 72 hours to become maximally elevated in response to either a relatively prolonged "stress" or ACTH medication, appearing to increase rather uniformly and continuously during this time interval. Of considerable interest has been the observation that in all cases of active rheumatoid arthritis and disseminated lupus erythematosus and in most cases of uncomplicated acute rheumatic fever thus far studied the leukocyte phosphatase activity has not been increased. This suggests that in these diseases and in these patients this form of "stress" may not result in increased endogenous pituitary and/or adrenal activity. These data are not adequately explained by alterations in the leukocyte differential formula in subjects studied. Further investigation anticipates exploration of time-dose relationships and the effect of other steroids.

Persistent Neutropenia with Maturation Arrest of the Bone Marrow in Graves's Disease, Not Related to Drugs. W. P. VANDERLAAN and HENRY W. COHEN, Boston, Mass. (Introduced by E. B. Astwood).

A woman presented classic features of Graves's disease, untreated, and a leukopenia. There were 3,100 leukocytes per cu. mm., 6% polynuclear neutrophils, 30% monocytes, 60% lymphocytes, 2% eosinophils, and 2% basophils. Bone marrow examination disclosed normocellularity, slight lymphocytosis, and maturation arrest at a promyelocyte level. Eosinophils were mature; there was no erythroid or megakaryocytic abnormality. The hematologic findings remained unchanged during one month during which there was no treatment. For two months after radioactive iodine treatment the hyperthyroidism and leukopenia persisted; the protein-bound iodine level was 15.6 μg./100 ml. In the next month both the hyperthyroidism and the leukopenia improved. Lymphocytosis, monocytosis, leukopenia, and anemia have been described in Graves's disease; the present case suggests that neutropenia approaching agranulocytosis may also be associated with thyrotoxicosis. Search disclosed no evidence of exposure to chemical agents known to suppress myelopoiesis.

Two-hundred and fifty ml. of the patient's plasma, taken after some improvement had occurred, was infused into a normal 42-pound boy and induced no significant change in his peripheral blood.

The case illustrates persistent neutropenia paralleling the severity of Graves's disease, not relating to drugs.

Turn-Over Rates of Various Plasma Proteins in Normal and Cirrhotic Humans Using Biosynthetic Methods. WADE VOLWILER,* PATRICK D. GOLDSWORTHY, MARION P. MACMARTIN, PATRICIA ANN WOOD, IAN R. MACKAY, KENNETH FREMONT-SMITH and DOROTHY F. SHOOK, Seattle, Wash.

With S-35 l-cystine, two contrasting biosynthetic techniques have been employed to measure turn-over rates of specific plasma protein fractions in normal subjects and in edema-free patients with advanced portal cirrhosis: (1) oral single administration of labeled amino acid precursor with subsequent serial sampling of plasma proteins for measuring rate of isotope decline, (2) transfusion of fresh normal pre-labeled donor plasma into suitable recipients, followed by measurement in the recipient of rate of loss of isotope from the infused proteins.

A total of 30 such determinations for specific protein fractions have been made, including albumin, β_1 lipoprotein and fibrinogen. Albumin has been prepared by the Batchelor-Brown modification of Cohn's Method 10, β_1 lipoprotein by Cohn's Method 10 with subsequent ultracentrifugation (Gofman), and fibrinogen by the method of Surgenor et al. The composition of these fractions from each experimental subject has been repeatedly appraised by electrophoresis, and homogeneity has varied from 80–100%.

Following are the average values of half-life (days) obtained for each fraction with biosynthetic techniques (1) and (2): Albumin: Normal (1) 29 (2) 20, Cirrhosis (1) 21 (2) 24; Cohn I + III: Normal (1) 7, Cirrhosis (1) 10; β_1 lipoprotein: Normal (1) 4.5 (2) 3.5, Cirrhosis (1) 7.7 (2) 5; Fibrinogen: Normal (1) 5 (2) 4, Cirrhosis (2) 4.

These data suggest: (1) Re-utilization of isotope does not produce error in measurement of half-lives with biosynthetic method I, (2) An abnormal size in the circulating mass of a specific plasma protein is not necessarily associated with an abnormal life-span, (3) Iodinization of plasma proteins may sufficiently denature protein as to result in a shortened life-span.

Evidence for a Qualitative Difference Between the Gamma Globulin of Patients with Multiple Myeloma and of the Normal Human. ROBERT L. WALL and LILLA SHU YUNG SUN, Columbus, O. (Introduced by Charles A. Doan).

Many investigators have postulated that the hypergammaglobulinemia of some multiple myeloma patients represents a protein that differs *qualitatively* from normal gamma globulin. Proof that such a difference exists has been lacking. In the present study evidence is presented to support such a qualitative difference.

Gamma globulin preparations of *adequate homogeneity* could not be obtained with existing fractionation procedures. Therefore, a method was developed employing zinc diglycinate as a precipitating agent with subsequent chelation of the protein-bound zinc by the sodium salts of ethylene-diamine-tetra-acetic acid. This method rou-

tinely produces an extremely *homogeneous* gamma globulin preparation as judged by the following tests: electrophoresis (both zone and boundary methods) with calculation of heterogeneity constants; optical ultracentrifugation with calculation of sedimentation constants; and by quantitative precipitin experiments employing specific rabbit antiserums. The method is applicable both to normal plasma and to plasma from myeloma patients.

Gamma globulin prepared by this method from pooled normal plasmas and from myeloma plasmas was then utilized for the production of rabbit antiserums. Previous quantitative precipitin experiments by the present authors, as well as other investigators, have shown that normal gamma globulin behaves as an immunologically homogeneous protein. Quantitative precipitin experiments with the present normal gamma globulin antiserum show marked differences in the precipitin curves when normal globulin and myeloma gamma globulin are used comparatively as antigens, as would occur if these proteins differed qualitatively. The reaction of myeloma gamma globulin antiserums with normal gamma globulin showed the presence of some normal gamma globulin in the myeloma gamma globulin. However, after maximal precipitation of the myeloma antiserum by addition of normal gamma globulin, this antiserum is still capable of precipitation with myeloma gamma globulin, demonstrating a true qualitative difference between normal and myeloma gamma globulin.

Hepatic Coma: Effect of Sodium Glutamate. LESLIE T. WEBSTER, JR. and CHARLES S. DAVIDSON,* Boston, Mass.

Ammonia or related compounds have been implicated as possible etiologic factors in the production of hepatic coma. The highest blood and plasma "ammonia" concentrations have been found in this condition. The syndrome of impending hepatic coma can be produced in some patients with hepatic disease by administration of ammonium salts. Glutamic acid plays an important role in intermediary nitrogen metabolism and can combine with ammonia to form less toxic compounds. Walshe reported favorable results with this amino acid in the treatment of hepatic coma.

In the present study 23 grams of sodium glutamate was given intravenously to eight patients with Laennec's cirrhosis who were in coma or impending coma. Patients evaluated daily with respect to state of consciousness and the characteristic "flapping" tremor were separated into three groups: coma, impending coma, and impending coma induced by ammonium salts. Three patients were studied on more than one occasion and in more than one group. Plasma "ammonia" concentrations were measured.

Two comatose patients showed questionable temporary improvement, one after three isolated infusions and the other after four daily infusions of glutamate; both subsequently died. Six patients with spontaneous impending coma received from one to three daily infusions of glutamate; again, with little, if any, improvement. Four in this group subsequently expired. Impending hepatic coma was induced in two patients by administration of ammonium salts. Glutamate failed to modify this syndrome which, however, disappeared after discontinuing ammonium salts. One of these patients was then given glutamate prior to ammonium salt administration, but this amino acid failed to prevent the onset of the characteristic syndrome.

Thus, sodium glutamate was found to have little, if any, beneficial effect on liver coma or impending coma; it also failed to prevent onset and exacerbation of impending coma induced by ammonium salts. Plasma "ammonia" concentrations showed no consistent changes.

Comparative Incorporation of S^{35} l-Cystine and S^{35} Sodium Sulfate by Normal and Leukemic Leukocytes. AUSTIN S. WEISBERGER, LEIF G. SUHRLAND and BENNETT LEVINE, Cleveland, O. (Introduced by T. H. Ham).

Previous studies indicate that *l*-cystine (or *l*-cysteine) may have an important role in leukocyte metabolism. Comparison of the uptake of S^{35} sodium sulfate with that of S^{35} *l*-cystine by normal and leukemic leukocytes reveals certain differences in incorporation both *in vivo* and *in vitro*.

Tracer amounts of S^{35} *l*-cystine or S^{35} sodium sulfate were administered orally to normal and leukemic patients. The concentration of S^{35} per ml packed leukocytes was determined at intervals. *In vitro* studies were obtained by incubating whole blood with these compounds and measuring the radioactivity incorporated per ml packed leukocytes.

Characteristic patterns of incorporation were obtained for orally administered *l*-cystine in normal and leukemic leukocytes. Acute leukemic leukocytes incorporated *l*-cystine most rapidly. The disappearance curves of radioactivity indicate that the approximate life span of leukocytes in normals is 13 days, in chronic myeloid leukemia 8 days, in acute leukemia 3 days and in chronic lymphatic leukemia longer than the period of observation.

Orally administered S^{35} sodium sulfate is utilized poorly by leukocytes in acute leukemia and in chronic lymphatic leukemia. However, incorporation of sodium sulfate by normal and chronic myeloid leukemic leukocytes is comparable to that of orally administered *l*-cystine.

l-cystine is readily incorporated by both normal and leukemic leukocytes *in vitro*. This is an active process and not solely dependent upon simple diffusion. In contrast, however, only chronic myeloid leukemic leukocytes readily incorporate sodium sulfate. All other types of leukocytes exhibit a low level of incorporation of sodium sulfate.

The data demonstrate differences in utilization of *l*-cystine and inorganic sulfur by normal and leukemic leukocytes. The findings are compatible with the apparently unique role of *l*-cystine in leukocyte metabolism.

A Study of Renal Tubular Phenomena Under the Influence of a Carbonic Anhydrase Inhibitor. L. G. WELT,* D. T. YOUNG, O. A. THORUP, JR. and C. H. BURNETT,* Chapel Hill, N. C.

The consequences of the administration of a carbonic anhydrase inhibitor were studied in three normal male subjects in an experimental setting characterized by a modest acidosis and a state of physiologic diabetes insipidus. The former was induced by the ingestion of ammonium chloride for five days, and the latter by the administration of an infusion of a dilute solution of glucose in water in a manner designed to achieve and maintain a positive balance of water of a liter or more. The carbonic anhydrase inhibitor promoted: 1) a striking increase in the rates of excretion of water, sodium, bicarbonate, and total solutes; 2) lesser increases in the rates of excretion of potassium and chloride; 3) decreases in the rates of excretion of ammonia and titratable acid; 4) an increase in the pH of the urine; and 5) a slight decrease in the clearance of inulin.

An increase in urine flow (despite a decrease in GFR) superimposed on a state of physiologic diabetes insipidus implies that a larger volume of fluid had gained access to the distal tubule. According to current concepts this would result from a decrease in active proximal reabsorption of solutes which, in this instance, were predominantly sodium and bicarbonate. Since these alterations were promoted by the administration of carbonic anhydrase inhibitor and the reabsorption of bicarbonate appears to be dependent on the secretion of hydrogen ions, the inference is that hydrogen ions are usually secreted in the proximal as well as the distal tubule.

The increase in urine flow was composed of increments of both "free" and "osmolar" water. These data can be explained as the result of an augmented distal tubular reabsorption of solutes consequent to the delivery of a larger volume of isosmotic fluid to this portion of the nephron.

SPCA-Induced Thrombosis: Its Pathogenesis and the Effect of Anticoagulants. STANFORD WESSLER, Boston, Mass. (Introduced by Herrman L. Blumgart).

The pathogenesis of thromboembolism and the relative efficacy of drugs in the prevention of intravascular coagulation remain poorly defined. In this laboratory an experimental method has been developed whereby, in dogs, the systemic infusion of serum fractions has routinely produced venous thrombosis under conditions of simple stasis. The role of anticoagulants in inhibiting these thrombi has also been investigated.

Serum fractions prepared by barium sulfate adsorption and citrate elution are rich in SPCA (convertin) and its precursor, pro-SPCA (proconvertin) but are devoid of significant amounts of prothrombin, thrombin, thromboplastin and Ac-globulin. Antecubital vein infusions of these SPCA preparations routinely induced rapid thrombus formation in jugular vein segments completely isolated immediately after infusion, although no measurable rise in pro-SPCA or SPCA was demonstrable. Direct venous narrowing or external application of tourniquets to intact extremities immediately after SPCA infusions similarly induced coagulation distal to the obstructed areas.

Dicumarol was given to ten animals with consequent reduction of one-stage prothrombic activity to two percent of normal or below. In only one dog was SPCA-induced thrombosis prevented; in this animal the coagulation defect was so severe that the clotting time had been tripled. In striking contrast, heparin administered to ten animals in amounts sufficient to double the clotting time produced complete inhibition of SPCA-induced clots in all instances.

Excessive SPCA activity has been observed in some clinical states and its possible relation to thromboembolism has been proposed. The studies herein described provide experimental evidence for the concept that minute amounts of *active* SPCA (contrasted with inactive pro-SPCA) may under conditions of simple stasis initiate extensive intravascular clotting. The data also demonstrate that SPCA-induced thrombi are more effectively inhibited by heparin than by Dicumarol even when the latter drug produces a profound reduction in the circulating SPCA complex.

Metabolic Studies Demonstrating Increased Resistance of Hyperthyroid Patients to Pitressin Tannate Administration. RAYMOND E. WESTON,* HELEN B. HOROWITZ, SHIRLEY RUBLER and JACOB GROSSMAN, New York, N. Y.

Previous studies have established that intramuscular administration of 1 unit of Pitressin Tannate-in-Oil (PTO) b.i.d. to normal subjects and patients in congestive failure, on adequate fluid intakes, leads to marked water retention, increased urinary total solute concentration (U.T.S.C.), and hyponatremia. In contrast, we found that 5 units of PTO t.i.d. had no metabolic effect in a severely hyperthyroid woman without demonstrable renal disease, maintained on a high salt diet.

Since then, three additional hyperthyroid women on our metabolic ward have been given PTO for 6–12 days. To evaluate the influence of urinary solute load, two were maintained on a high (175 m.Eq.) and one on a low (25 m.Eq.) sodium intake. To compensate for hyperidrosis, fluid intakes were considerably increased.

All exhibited resistance to Pitressin. On 2 units t.i.d., one patient on high salt intake exhibited transitory increase in calculated U.T.S.C. from 366 m.Osm./l. to 450–520 m.Osm./l., without change in urine volume (1800–2400 ml.) or serum electrolyte concentrations. Because of severe hyperidrosis, another on high salt intake had control urine volumes of 1400–1600 ml. with measured U.T.S.C. of 564–608 m.Osm./l. On PTO (2 units t.i.d.) her U.T.S.C. rose slightly to 650–700 m.Osm/l., and her serum sodium fell 7 m.Eq./l., partly due to increased natriuresis on the sixth and last day. The following day,

her urine volume increased to 2200 ml., U.T.S.C. dropped transiently to 390 m.Osm./l. weight fell 1 kgm., and serum electrolytes returned to normal. The one patient on the low sodium intake exhibited only slight antidiuretic response to PTO (2 units t.i.d.), as indicated by small U.T.S.C. increases from 285–355 m.Osm./l. to 365–420 m.Osm./l., with some water retention and subsequent natriuresis and hyponatremia.

Possible mechanisms responsible for the apparent resistance of these hyperthyroid patients to the anti-diuretic effect of PTO will be discussed.

Clinical Studies on Staphylococcal Septicemia. ROBERT I. WISE and CAROLINE CRANNY, Minneapolis, Minn. (Introduced by Wesley W. Spink).

Fifty-one cases of severe staphylococcal septicemia were studied and treated at the University of Minnesota and associated hospitals from November, 1951, through February, 1954. Twenty-one patients recovered, 27 expired and 3 are under treatment. The mortality rate, thus far, is 53%. Sensitivity of the cultures of *Micrococcus pyogenes var. aureus* (Staphylococci) to the antibiotics was studied *in vitro* by the tube-dilution technique.

The percentages of cultures which were resistant to 50 units or micrograms per ml. were as follows: penicillin 70.1%, streptomycin 65.7%, chlortetracycline 37.2%, oxytetracycline 46.6%, and chloramphenicol 2.5%. The strains were sensitive to bacitracin, erythromycin, carbomycin and neomycin at concentrations of less than 10 units or micrograms per ml. in percentages of 92%, 100%, 97%, and 82% respectively. The percentages of cultures sensitive to one unit or microgram per ml. were as follows: penicillin 17.1%, streptomycin 2.4%, chlortetracycline 21%, oxytetracycline 23.1%, chloramphenicol 0%, bacitracin 26.2%, erythromycin 87.8%, and neomycin 34.2%.

Bacteriophage typing of the cultures revealed 8.2% in group II, 44.5% in group III, 5.6% in a miscellaneous group (type 44A), none in group I, and 41.7% were non-typeable. Antibiotic-resistant cultures were prominent in group III and the non-typeable group. The percentages of group III strains resistant to 50 units or micrograms per ml. were as follows: penicillin 87.5%, streptomycin 75%, chlortetracycline and oxytetracycline 43.7%. The percentages of non-typeable strains resistant to 50 units or micrograms per ml. were as follows: penicillin 66%, streptomycin 73%, chlortetracycline 33% and oxytetracycline 53%. Strains in group II and the miscellaneous group were sensitive or slightly resistant to all antibiotics. No correlation existed between the bacteriophage types and death of the patients.

Patients surviving were treated with combinations of antibiotics, which were selected on the basis of *in vitro* sensitivity tests. The combinations included penicillin and bacitracin, erythromycin and bacitracin, or carbomycin and bacitracin.

Oxygen Content of Pulmonary Artery Blood During the Respiratory and Cardiac Cycle in Relation to Possible Errors in Flow Measurement by the Fick Method. EARL H. WOOD* and JOHN T. SHEPHERD, Rochester, Minn.

Changes in blood flow and oxygen content associated with respiratory or cardiac cycles may introduce large systematic errors in determinations of cardiac output (Visscher). To study this possibility, a method was devised for withdrawal of blood samples from the heart and great vessels during any phase of respiratory or cardiac cycles. This consists of a synchronizer circuit controlling an electromagnetic valve interposed between cardiac catheter and blood-sampling syringe. Delay and hold circuits allow the synchronizer when activated by the onset of inspiration or expiration or by the R wave of the ECG to hold the sampling valve open during any portion of respiratory or cardiac cycles. Oxygen saturation of blood withdrawn in this manner was determined by photometry and manometric methods. In 8 subjects without shunts (2 normal and 6 with mitral disease) there was no demonstrable difference in oxygen content of pulmonary artery blood during inspiratory or expiratory phases of the respiratory cycle. In 4 subjects (2 normals) there was no difference during systole and diastole. Failure to demonstrate differences means that if cyclic variations did occur, they were small. Hence, possible errors from this cause in cardiac output values by conventional Fick methods were also probably small.

Phasic differences in oxygen content of pulmonary artery blood were not demonstrated in 4 patients with large left-to-right shunts via atrial septal defects. However, small but probably significant changes in pulmonary artery samples with the cardiac cycle were found in some patients with patent ductus arteriosus or ventricular septal defect. The direction and magnitude of the systematic error in determination of pulmonary blood flow by the conventional Fick method under such circumstances is related to the product of the amplitude of the variations and the cosine of the phase angle between the variations in flow and oxygen content.

Quantitative Fractionation of Serum Proteins Determined by Moving Boundary and Paper Electrophoresis. MOSES WURM, New York, N. Y. (Introduced by Ernst P. Boas).

Paper electrophoresis gives satisfactory *qualitative* separations of a variety of complex mixtures but the *quantitative* aspects of the method as applied to serum proteins have not been adequately examined. To obtain consistently reproducible electrophoretic patterns that lend themselves to quantitation it was essential to keep the filter paper strips freely suspended in a taut, horizontal position throughout the period of electrophoresis and subsequent drying. Staining either with bromphenol blue or Amido-Schwarz 10B gave similar values. The dry patterns were measured in a photoelectric densitometer (Photovolt 501A) using white light, and curves were obtained by plotting the antilog of the optical den-

sity readings against distance of migration on the paper strip. This conversion expressed the observed proportionality, determined from calibration curves, between optical density and the logarithm of protein concentration.

Serum protein distributions thus determined compared favorably with the values obtained in a moving boundary apparatus (Aminco Portable). Ten pairs of consecutive determinations on the serum of a single donor by paper (P) and moving boundary (M) electrophoresis expressed in terms of relative protein concentrations gave the following mean results ± S.E.: albumin (P) 58.5 ± 1.29 and (M) 59.1 ± .67 (t = .41); alpha$_1$-globulin (P) 3.9 ± .27 and (M) 4.5 ± .24 (t = 1.39); alpha$_2$-globulin (P) 7.2 ± .27 and (M) 8.7 ± .47 (t = 2.77); beta-globulin (P) 13.0 ± .56 and (M) 13.9 ± .35 (t = 1.36); gamma-globulin (P) 17.4 ± .94 and (M) 13.8 ± .44 (t = 3.46). Statistically, the albumin values showed a high degree of identity while the alpha$_1$-globulin and beta-globulin values were at variance within experimental limits. The highly significant difference in gamma-globulin values might be attributed to protein adsorption on paper, an inherent characteristic of paper electrophoresis. The significant difference between the alpha$_2$-globulin values remains unexplained.

Excretion of Hydroxyproline in Patients with Rheumatic Diseases and in Children. MORRIS ZIFF, ANDRE KIBRICK, ELLIS DRESNER and H. JOEL GRIBETZ, New York, N. Y. (Introduced by Currier McEwen).

Hydroxyproline constitutes 15 per cent of collagen, and except for small amounts present in elastin, is found in no other protein of the body. The excretion of this amino acid was determined in patients with "collagen diseases" as well as in control subjects in order to determine whether differences due to abnormal collagen metabolism might be detected. Forty-five adults and fourteen children were placed on a standard low hydroxyproline diet. Free and total urinary oxyproline were determined by the colorimetric method of Wiss, which was shown to agree closely with a chromatographic, radioactive isotope derivatization method. Total amino acid, creatinine, and nitrogen were also determined.

Over 95 per cent of excreted hydroxyproline was in bound form. Excretion was not increased by administration of cortisone or by ingestion of 4 grams of oxyproline or proline daily, although there was a prolonged rise in blood level and in excretion of free hydroxyproline after ingestion of the latter. Hydrolysis studies suggest that the bound hydroxyproline of urine is in peptide linkage. Since this peptide is apparently not formed from administered hydroxyproline, it may represent a breakdown product of collagen.

No significant difference was observed between the excretion of hydroxyproline in normal adults and in a group of adult patients with miscellaneous diseases on the one hand, and patients with rheumatoid arthritis and other "collagen diseases" on the other, either on an absolute basis or as per cent of total amino acid. Thus no significantly increased breakdown of collagen in patients with diseases of the connective tissue was demonstrated.

The excretion of hydroxyproline in children was two to three times greater than in adults on an absolute basis, and four to five times greater when expressed as per cent of total amino acid. Increased excretion of bound hydroxyproline by children may indicate the presence of an increased pool of hydroxyproline peptide for collagen synthesis in the growing individual.

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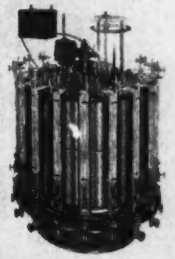

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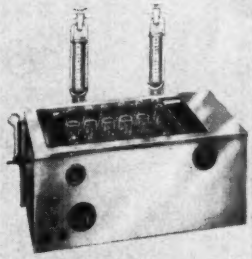

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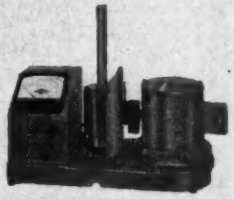

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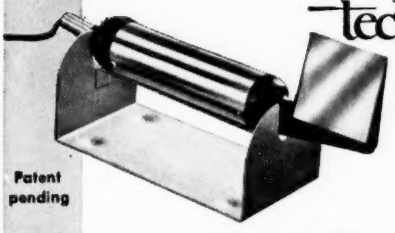

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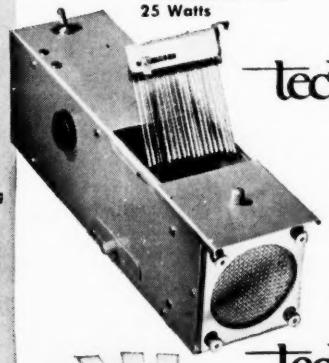

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Retarded absorption of the injected insulin has been postulated as the source of the problem. Gysin and Wilson,[1] among others,[2,3] appear to have demonstrated such a mechanism by their success with hyaluronidase as an adjunct to insulin. Hyaluronidase hydrolyzes hyaluronic acid, the intercellular material that normally resists the diffusion of intrusive substances. This specific property of hyaluronidase increases tissue permeability, and is used clinically to accelerate the transit of injected drugs to the capillaries.

SCOPE OF INVESTIGATION

Gysin and Wilson's studies were undertaken to determine whether hyaluronidase would provide three important advantages to the induction of insulin coma:

1. Earlier and more complete absorption of insulin, with production of therapeutically adequate coma
2. Reduction in the dose of insulin, with a corresponding increase in the margin of safety
3. Reduction in delayed effects due to continued absorption of insulin after termination of coma

METHOD

Each of the 53 patients in the series served as his own control. The administration of insulin alone was alternated throughout the course with the combined administration of hyaluronidase and insulin, each phase lasting for from 10 to 15 comas. Wydase (lyophilized hyaluronidase, Wyeth) was preferred to other brands because of its stability and because the 10-cc. vials (1500 turbidity-reducing units) permit the preparation of a large stock of solution at one time. Experience showed that a dose of 0.5 cc. (75 TRU) of Wydase solution with each injection of insulin was as satisfactory as larger amounts. It was also discovered that the size of the insulin dose made little difference in the amount of Wydase required for effective absorption.

RESULTS

1. Wydase with insulin improved the quality of the coma, and promoted smoother induction, with less agitation, and more rapid progression through the precoma to the coma phase.

2. With Wydase, less insulin was required for satisfactory coma. The reduction in dose averaged from 35 to 40 per cent throughout the series. In 9 patients, the amount of insulin was reduced by from 50 to 73 per cent.

3. Late adverse reactions were reduced by about 73 per cent, since with Wydase the accelerated absorption of insulin tended to confine the effects to the morning hours.

4. Of the 53 patients, 5 remained refractory even when the amount of Wydase was increased to 2 cc. (300 TRU) for each injection of insulin. A sixth apparently refractory patient responded satisfactorily when 1.5 cc. (225 TRU) of Wydase was used.

5. There was no evidence of local or systemic toxicity or of hypersensitivity to Wydase.

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